

THE ALKALOIDS OF ARTABOTRYS SUAVEOLENS, Blume

(N.O. Anonaceae)

by

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Preface

This thesis embodies the results of a research carried out in the laboratory of Medical Chemistry of the University of Edinburgh during the academic years 1936-37, 1937-38, under the supervision of Professor G. Barger, F.R.S.

The author wishes to express his sincere thanks to Professor Barger for valuable advice and criticism as well as for his continued interest throughout the execution of this work.

The author desires also to thank Drs. E.G.Cox and C.J. Brown of the University of Birmingham for their X-ray analysis of one of the alkaloids; Dr M. Guggenheim of the Hoffmann-La Roche Co., Basel, for some pharmacological experiments; Dr E.B. Ludlam of the Chemistry Department, University of Edinburgh, for helpful suggestions in connection with the spectrographic work; Dr J.J. Blackie of Messrs Duncan, Flockhart and Co., Edinburgh, for the extraction of quantities of bark and leaves; the Moray Fund of the University of Edinburgh for grants which have defrayed the costs of materials.

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(a) Introduction.

Botanical and Pharmacological ⁽¹⁾

The Anonaceae are almost exclusively inhabitants of hot climates. They extend over the whole world for about 40° on each side of the equator; but in Africa they seldom pass 20° N. Europe is the only quarter of the world in which none are indigenous; the few species cultivated in the open being those from North America. The sections *Porcelia* and *Asimina* (of *Uvaria*) belong to the United States, Mexico, and the western regions of South America as far as Peru. The south-east of this American zone, as far as the south of Brazil is the country of most species of *Anona*.

Besides *Anona*, *Uvaria* and *Unona*, four other genera are common to both hemispheres: *Xylopia*, *Bocagea*, *Anaxagorea* and *Phaeanthus*. *Xylopia* is distributed over the largest geographical area, being represented in tropical Africa, Madagascar, India, Polynesia, Guinea, and as far south as Brazil. The genus *Bocagea* consists of several Brazilian species; *Phaeanthus* is disseminated over a wide area, one species coming from Brazil, two from tropical Africa and as many from the Indian Archipelago. *Anaxagorea* is/

is about equally divided between the tropical regions of Asia and America.

The genera *Popowia*, *Miliusa*, *Oxymitra* and *Artabotrys* occur in both tropical Asia and tropical Africa. *Artabotrys* also extends to China and the Philippines.

The uses of the plants of this order are numerous, especially in the warm regions where they grow abundantly. They are often aromatic, and consequently stimulant, stomachic, sometimes bitter, tonic, febrifuge and antiputrescent. But the exaggeration of these properties may also sometimes render their employment dangerous.

Several Asiatic *Unonas* and *Uvarias* are used as stimulant drugs. From their bark and pulp are prepared decoctions, applied locally for bruises and rheumatic pains, and administered as stomachics to facilitate digestion. Blume (*Flora. Java. Anonac.*) has pointed out that as drugs these barks are especially efficacious in affections arising from the portal vein, but that they must be used with caution, for in excess they produce vertigo, hæmorrhage and even abortion.

The/

The Natural Order Anonaceae comprises over 800 species, and although the more characteristic plant products, such as fatty and aromatic acid esters, aromatic alcohols, carbohydrates and essential oils, have been detected in, and isolated from, the leaves, fruits, flowers and seeds of most of the species, the presence of alkaloids has been demonstrated, with certainty, in only a few. The following table brings together those species of Anonaceae which are known to be alkaloid bearing.

Table I.

Species	Alkaloid	Reference
Anona squamosa, L.	"Anonaine" (C ₁₇ H ₁₇ O ₃ N)	Trimurti (2) Reyes and Santos (3)
Anona reticulata, L.	"Anonaine"	Santos (4)
Anona muricata, L.	Amorphous alkaloids (not investigated)	Callan and Tutin (5)
Anona triloba, L.	"Asiminine" (not investigated)	Lloyd (6) Fletcher (7)
Alphonsea ventricosa, Fried.	"Alphonseine" (not investigated)	Czapek (8)
Popowia piscarpa, Endl.	Toxic alkaloid (not investigated)	Boorsma (9)
Xylopia polycarpa, Oliv.	"Berberine" (?)	Stenhouse (10)
Phaeanthus/		

Table I contd.

Species	Alkaloid	Reference
<i>Phaeanthus ebracteolatus</i> , (Pres.)Merrill.	"Phaeanthine" ($C_{34}H_{38}O_6N_2$)	Santos(11)
<i>Artabotrys suaveolens</i> , Blume	"Artabotrine" ($C_{36}H_{55}O_6N$) ($C_{21}H_{25}O_4N$)	Greshoff(12) Maranon(13) Santos(14) De Rochebrune(15)

Before proceeding to a discussion of the chemistry of the alkaloids derived from the Anonaceae, it would perhaps be of interest to know something of the species which have been responsible for these alkaloids. It has been mentioned above that the Anonaceae are cultivated in nearly all the warm countries of the earth; moreover, several species of this family bear fruits that are often prized as aliments or drugs. One of the best known is the fruit of:

(1) Anona squamosa, L. (the sweet sop or sugar apple), a native of the Antilles, cultivated for its fruit in all the tropical regions of both hemispheres. Its perfume is sweet and its taste agreeable. The young fruit is astringent, and the seeds acrid; these when powdered are used to destroy vermin. Trimurti(2) first reported the presence of an alkaloid in both the leaves and seeds of this species. Although he prepared/

prepared both the hydrochloride and chlorplatinate, he was unable to crystallize the free base, obtaining it, instead, in the form of a white powder. The actual crystallization of the base was achieved somewhat later by Reyes and Santos (3), who showed that this alkaloid was identical with "Anonaine" which they isolated from *A. reticulata*.

(2) Anona triloba, L. (also known as Asimina t.)

The fruit of the American species, sometimes called Monin or Papaw, is not of a very agreeable taste. Nevertheless an alcoholic drink can be obtained from it. The pulp and bruised leaves are applied to ulcers, to induce cicatrization, and to abscesses, whose maturation it is supposed to hasten. The seeds are acrid, like those of many Anonaceae; reduced to powder they are used as an emetic, or to destroy vermin. From the seeds of this species, Lloyd(6) and somewhat later, Fletcher (7) isolated an amorphous alkaloid to which the name "Asiminine" was given. Other than the demonstration of the presence of an alkaloid, nothing further was reported.

(3) The fruit of Anona muricata, L. (the sour sop), is cultivated in Arabia, and, when ripe, is supposed to be an anti-scorbutic and febrifuge; moreover, picked/

picked before maturity, dried and powdered, it is administered in cases of dysentery, after the inflammatory symptoms have been removed by appropriate treatment. The seeds are astringent, and from the leaves are prepared poultices. Callan and Tutin(5) from an examination of a small quantity of leaves at their disposal found that, among other things, they yielded a small amount of an amorphous substance which gave positive tests with most alkaloid reagents. The substance, however, was not further investigated.

(4) Anona reticulata, L., a fruit-bearing tree cultivated in Mauritius, East India and Brazil, is the source of the custard-apple which, although edible, is not much esteemed. The leaves have a strong narcotic odour; and the juice that flows from the cut branches is acrid, and inflames the conjunctiva if dropped into the eye. As a drug, the green fruit and leaves are employed just like those of A. muricata, above.

From the bark of this tree, Santos (4) isolated a crystalline non-phenolic alkaloid for which he suggested the name "Anonaine". He was able to show a little later on (3), that his anonaine was identical with the alkaloid present in A. squamosa which, although investigated earlier by Trimurti (loc. cit.) had/

had not previously been obtained in crystalline form. From the analysis of two salts of the base, Santos was able to ascribe the formula $C_{17}H_{17}O_3N$ to anonaine.

(5) The fruits of many Xylopias are used as aromatics. The one longest known for this is the Guinea pepper, the berry of X. aethiopica. Guinea pepper has been used as a drug (i.e. as an intestinal stimulant), and the negroes have employed it as a condiment from time immemorial.

In the pharmacies of Brazil, the fruits of three of these species are to be found, viz. X. grandiflora, X. sericea, and X. frutescens. All are energetic tonics for the stomach and the intestines on which they have a binding, carminative and stimulant action.

According to Stenhouse (10), the bark of the West African X. Polycarpa, Oliv. (= Coelocline p.D.C.) contains the alkaloid berberine. However, since there was some uncertainty in this matter, Klein and Bartosch (16) recently re-examined the plant and failed to detect the presence of any berberine. The bark is rich in a yellow dye, and it is conceivable that this fact may have had something to do with Stenhouse's suggesting the presence of berberine.

(6)/

(6) The Alphonsea, is a rather small genus of the Anonaceae family, consisting of small trees and shrubs found from north-eastern India and southern China southwards to Ceylon and Malay-Asia. The leaves of A. ventricosa, (Hook.f. and Thoms), contain 0.5% of the poisonous alkaloid "Alphonseine" (8), which has not, as yet, been investigated.

(7) Phaeanthus, Hook.f., comprises a small genus of trees found in Tenasserim, Annam, the Andaman Islands to Sumatra, the Philippines and eastwards to New Guinea. The Philippine species, P. ebracteolatus, (Pres.) Merrill, deserves mention here for two reasons: first, because of its employment by the natives to alleviate inflammation of the eye, and second, because of its relatively high alkaloid content, 0.7%. According to Santos (11), the bark of this species contains at least two alkaloids: one of which is tertiary and non-phenolic, whereas the other appears to be a quaternary base. Only the former has thus far been investigated and, for this alkaloid, the name "Phaeanthine" was suggested. Analysis of the crystalline base gave rise to the empirical formula $C_{35}H_{40}O_6N_2$, whereas the formula $C_{34}H_{38}O_6N_2$ was found to be in better agreement with analytical results obtained with salts of the base. It/

It still remains to be determined which of the two formulae is the correct one.

(8) The bark of Popowia pisocarpa, Endl., a tree commonly cultivated in Java for its durable wood, was shown by Boorsma (9) to contain a small amount of a weakly toxic, crystalline alkaloid. This has not been further investigated.

(9) The species Artabotrys suaveolens, Blume, appears to be widely disseminated. It is found from eastern Bengal throughout Burma and Malay-Asia; it is also abundant in the East Indies, southern China and in the Philippine Islands.

In the Philippines - the habitat of the species used in this investigation - the plant is a considerable woody climber, and occurs in dry thickets, and in second-growth forests at low altitudes. Among the natives, the plant is usually known as Báhaibalagán; kintubó; súsong or damúlag.

In general the flowers of Artabotrys are very aromatic, and several species of the genus afford a scented oil which is much used as a perfume in Java. Several of the species also bear edible fruits, and the young leaves are eaten by cattle. In India(1) an aromatic infusion is made from the old leaves for the treatment of cholera. Among the many Philippine species/

species, A. odoratissimus, R. Brown, and A. suaveolens, Blume contain active principles which act as stimulants when administered in therapeutic doses. If, however, the doses are abnormally increased, haemorrhage and nervous disturbances may result.

Up until 1929, when Maranon (13) re-examined the species, little had been published concerning the presence of alkaloids in this plant; the only reports then existent were the very meagre ones by Greshoff (12), and by de Rochebrune (15). The former pointed out that the bark of the branches, but not the leaves, of A. suaveolens from the Dutch East Indies contained about 0.1% of an alkaloid accompanied by a blue fluorescent substance. From some local samples of bark (Philippines) from the stems and roots of A. suaveolens, Maranon (loc. cit.) succeeded in isolating in pure form, a small quantity of crystalline alkaloid of m.p. 187° , which he named "Artabotrine" and, from his analysis, an empirical formula $C_{36}H_{55}O_6N$ was assigned to it. He also prepared the crystalline hydrochloride and hydrobromide of the base, and found that a few mg. of the hydrobromide, when injected subcutaneously, were fatal to a guinea-pig. The pronounced external symptoms were difficulty in respiration, stretching of the legs, dilatation of the pupil of the eye and convulsion.

Santos and Reyes (14), upon reinvestigating the problem, found Maronon's formula ($C_{36}H_{55}O_6N$) to be in error, and suggested instead the formula, $C_{21}H_{25}O_4N$. In addition, these authors reported the isolation of a new alkaloid, phenolic in nature and of m.p. 182° . For this substance the name "Suaveoline" was suggested, and from analyses they assigned to it the empirical formula, $C_{20}H_{23}O_4N$.

(b) The Origin of Alkaloids in Plants.

The view that the primary products of assimilation, in plants, are the same for the synthesis of proteins and for alkaloids, was expressed by Gadamer (17) almost twenty-five years ago. When assimilation is intense, he suggests, alkaloids are produced, but during periods of diminished assimilation the enzymes which synthesize proteins may break down the alkaloids, the disruption products serving in the formation of the proteins.

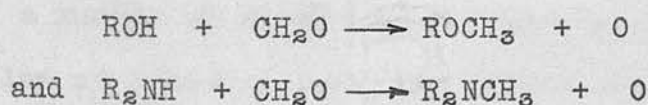
Yet an earlier, but more substantial theory regarding the formation of alkaloids in plants is the one due to Pictet (18) who suggested that alkaloids are waste products and are produced in plants in two successive stages, involving:

(1) the disruption of complex nitrogenous substances, such/

such as protein or chlorophyll with the production of comparatively simple basic products, and (ii) the recombination, or condensation of these simple basic products with other substances already present in the plant, with the resulting formation of the complex structures found in alkaloids.

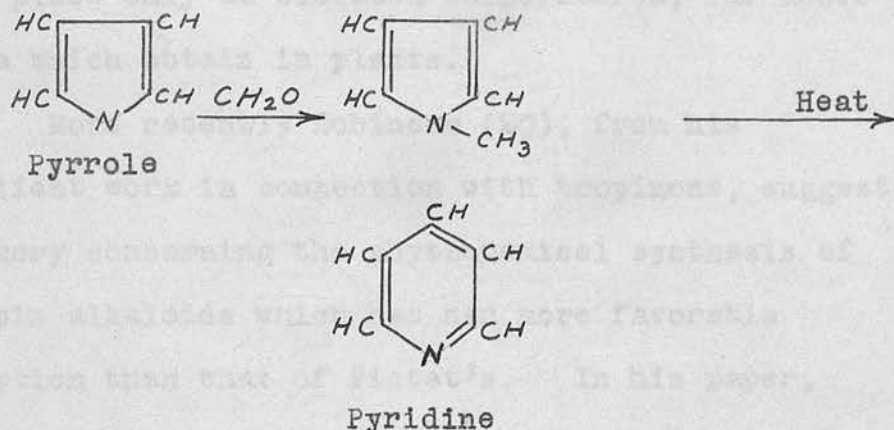
If the generally accepted view that alkaloids are waste products is true, then the metabolic processes in plants are very similar to those which obtain in the animal organism; in which simple waste products, such as p-cresol and glycolic acid are linked with other substances, such as sulphuric acid and benzoic acid to produce the more complicated structures p-cresol-sulphuric acid and hippuric acid respectively, in which form they are usually encountered in excreted matter.

In addition to the above, Pictet believed that among the most common changes which take place within the plant are the methylation of hydroxyl and imino groups by formaldehyde according to the equations:



The resulting methylated compounds are then able to undergo intra-molecular transformation, by which the methyl/

methyl group can enter the ring, and so produce, for example, a pyridine ring from methyl pyrrole, a reaction which he succeeded in carrying out in the laboratory.



Since pyrrole and indole rings are found in the normal constituents of protein (e.g. proline, tryptophane), by a similar set of transformations, the origin of pyridine, quinoline and iso-quinoline rings could be accounted for.

As experimental evidence in support of these views, Pictet and Court (19) offered the isolation of a number of simple bases from the steam distillation of tobacco, coca and carrot leaves. They succeeded in identifying pyrrolidine and methyl pyrroline from the mixture of bases, and spoke of them as proto-alkaloids.

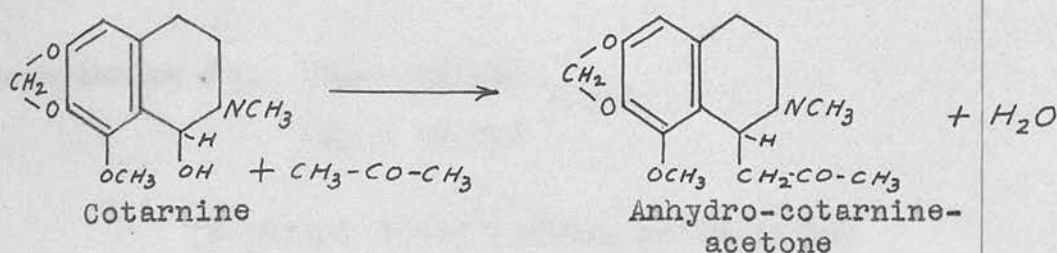
Although Pictet's original views on the methylating/

methylyating action of formaldehyde have been generally accepted, his ideas on the transformation of pyrrole to pyridine, etc. have not been favorably received. This is mainly because such reactions are known to take place only at elevated temperatures, far above those which obtain in plants.

More recently Robinson (20), from his brilliant work in connection with tropinone, suggested a theory concerning the phytochemical synthesis of certain alkaloids which has had more favorable reception than that of Pictet's. In his paper, Robinson points out that practically the whole of the work upon which his views are based, could be traced back to two important reactions, by means of which union between carbon and carbon could be brought about. In the first instance he lays stress on the aldol condensation, and in the second on the condensation of an aldehyde or ketone with ammonia or an amine to a carbinolamine, $\text{-}\overset{|}{\text{N}}\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-OH}$ and the subsequent reaction of this with substances containing the group $\text{-}\overset{|}{\text{CH}}\text{-CO-}$ to form: $\text{-}\overset{|}{\text{N}}\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-CO-}$.

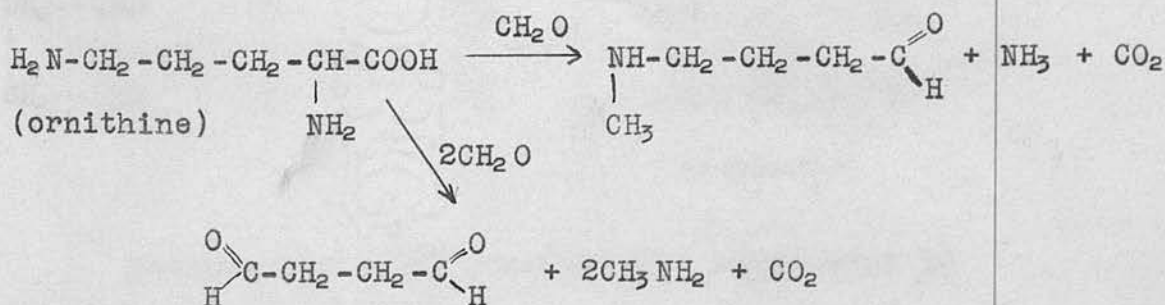
For example, he showed that the condensation of cotarnine with acetone to anhydro-cotarnine-acetone proceeded almost to completion in aqueous solution at room temperature, thus:



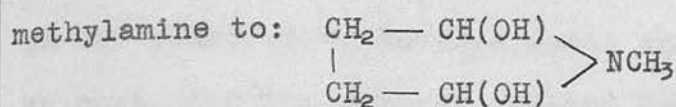


Since the starting materials for the phytochemical syntheses must come from the plant, the following substances, according to Robinson, are to be considered as most important, viz. ammonia, formaldehyde, ornithine, lysine and degradation products arising from carbohydrates, particularly citric acid which can give rise to acetone-dicarboxylic acid on oxidation, thus supplying the required acetone complex.

Robinson further pointed out, citing earlier work, that formaldehyde has both a methylating and oxidizing effect on amines, hence on amino acids as well, and by the following scheme indicated how succindialdehyde might arise from ornithine:

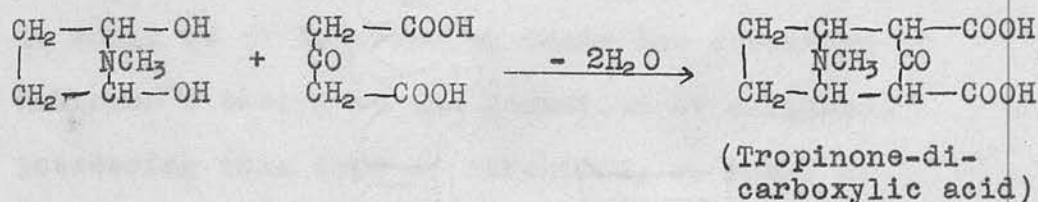


Succindialdehyde can undergo condensation with methylamine/



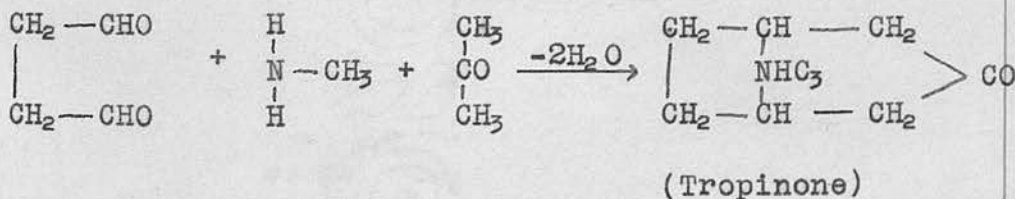
(N-methyl di- $\alpha\alpha'$ -hydroxy pyrrolidine)

and this on condensation with acetone-dicarboxylic acid would yield tropinone-dicarboxylic acid:



which on decarboxylation would give rise to tropinone.

To substantiate this hypothesis, Robinson was able to demonstrate beyond doubt that tropinone could be arrived at in a very similar manner. By mixing together in aqueous solution succindialdehyde, methylamine and acetone, and allowing the mixture to stand at room temperature, he was able to detect the presence of tropinone (characterized as its dipiperonylidene derivative) in half an hour.

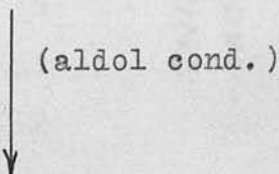
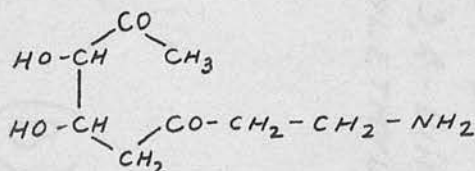
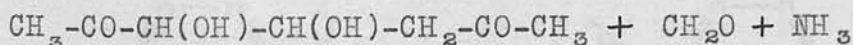
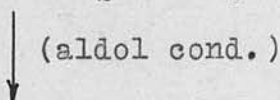
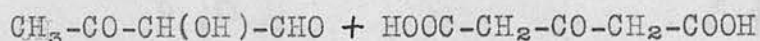


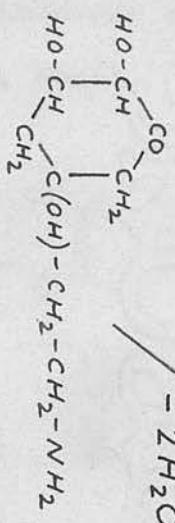
Because the conditions in this experiment so closely approximate those which are known to prevail in/

in the plant (i.e. aqueous media and low temperatures), we must, for the present, accept Robinson's view that the alkaloids are produced in plants by a similar series of reactions.

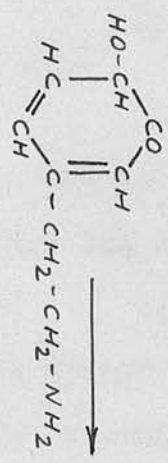
Since this thesis concerns itself with alkaloids possessing an iso-quinoline ring system, it would be of interest to trace the extension of Robinson's theory to the formation of alkaloids possessing this type of structure, by means of reactions which have already been indicated.

In his scheme for the synthesis of iso-quinoline alkaloids, Robinson starts with ammonia, formaldehyde, acetylglycollaldehyde and a reactive acetone derivative, as follows:





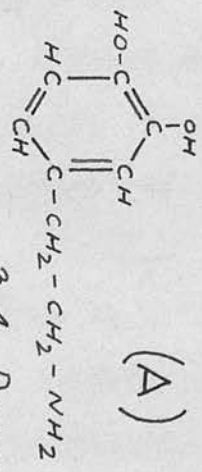
- 2H₂O



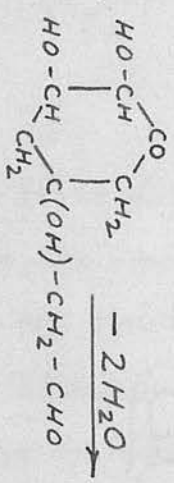
β-PHENYLETHYLAMINE

3,4-DIHYDROXY-

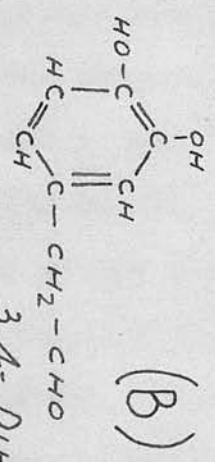
(A)



OXIDATION



- 2H₂O

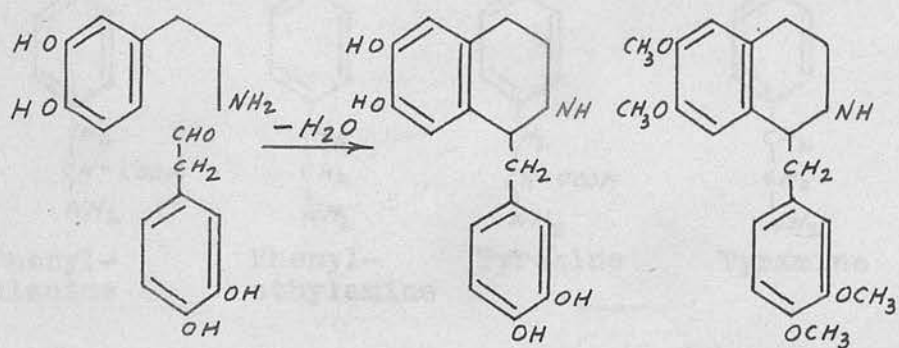


(B)

3,4-DIHYDROXY-

PHENYLACETALDEHYDE

A and B may condense, with the elimination of one mole of water, to a structure met with in alkaloids related to papaverine, thus:



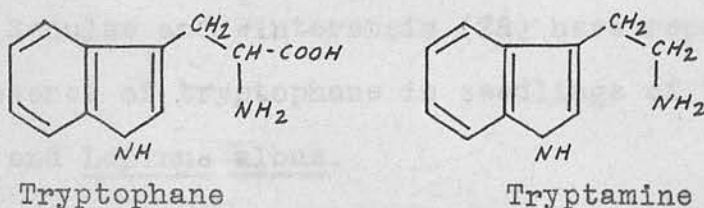
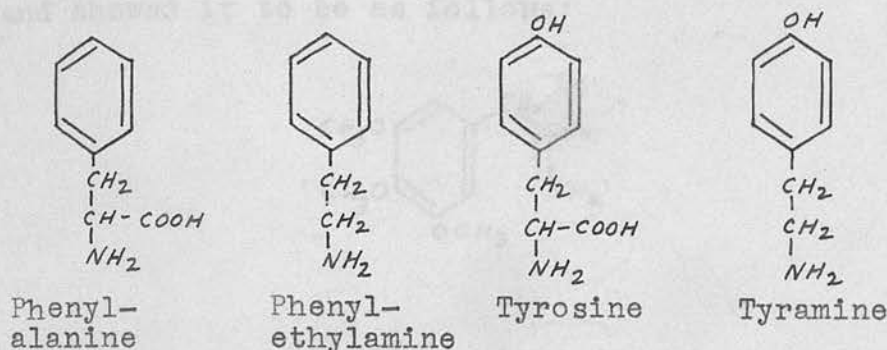
(nor-laudanosine)

It is interesting to note that nor-laudanosine has, in fact, been produced in this way by Spaeth and Berger (21).

Although, from the above, it is theoretically possible to account for the synthesis of complex ring systems starting with relatively simple aliphatic compounds, it is generally assumed to be more likely that the immediate precursors of the alkaloids result from amino acids present in the protein of the plant.

Specifically, such substances as tyrosine, tryptophane, phenylalanine and their decarboxylated analogues tyramine, tryptamine and phenylethylamine might well serve in this capacity. And this view becomes all the more interesting when we note that/

that several investigators have demonstrated the presence of these substances, or of their derivatives, in plants.



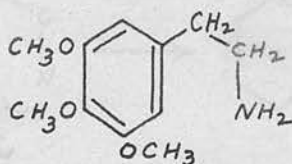
Thus, phenylalanine was discovered by Schulze and Barbieri (22) in etiolated germinating lupin seeds.

Tyrosine, according to Shibata(23) occurs in considerable quantity in rapidly growing shoots of Japanese bamboos; Schulze (24) has found it in small quantity in seedlings of Lupinus albus, and it has been described by Besanez (25) as occurring with asparagine in the root-tubers of Dahlia variabilis.

N-dimethyl tyramine, also known as hordenine, occurs in the embryo of barley, and its constitution was proved by the synthesis of Barger (26).

Moreover/

Moreover, from the cactus of the Anhalonium family, Spaeth (27) has isolated, among others, the base mezcaltine, whose constitution he proved by synthesis and showed it to be as follows:



o-methyl-dimethoxy tyramine

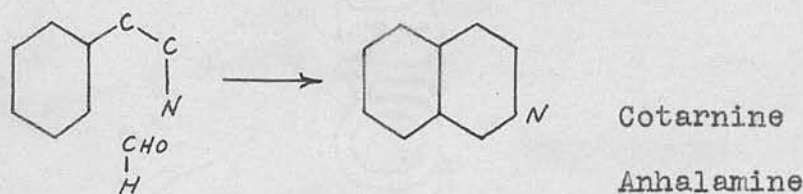
Schulze and Winterstein (28) have reported the presence of tryptophane in seedlings of Vicia sativa and Lupinus albus.

As was illustrated above, a substituted phenylethylamine can condense with a substituted phenylacetaldehyde which, as we have seen may arise from phenylethylamine as a result of the oxidizing action of formaldehyde, to a ring system of the papaverine type. Indeed, nearly all of the isoquinoline alkaloids may be accounted for by different ring closures which can occur between phenyl-ethylamine and formaldehyde, acetaldehyde or phenylacetaldehyde, and these may be classified as follows:

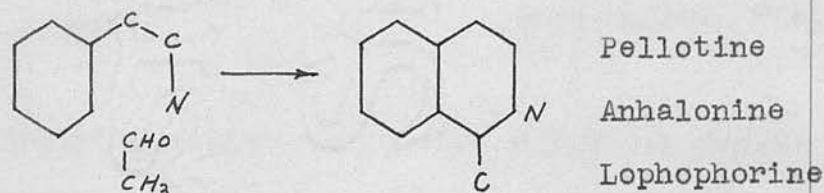
A/

A. Iso-quinolines arising from one tyrosine residue.

(i) Ring closure between mezcaline (o-methyl-dimethoxy tyramine) and formaldehyde leads to:

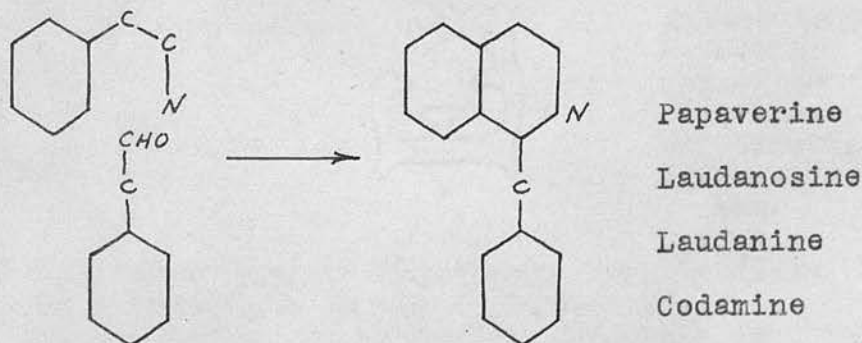


(ii) similarly with acetaldehyde:



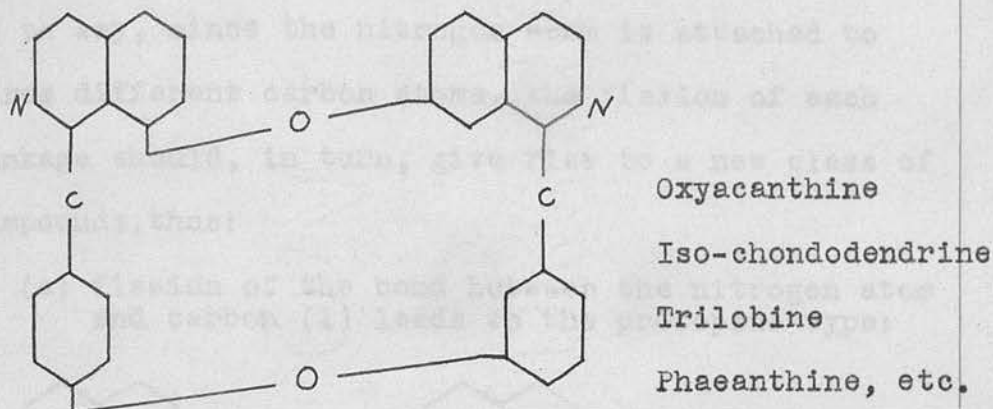
B. Iso-quinolines arising from two tyrosine residues.

(i) Ring closure between phenylethylamine and phenylacetaldehyde leads to the benzyliso-quinoline type:



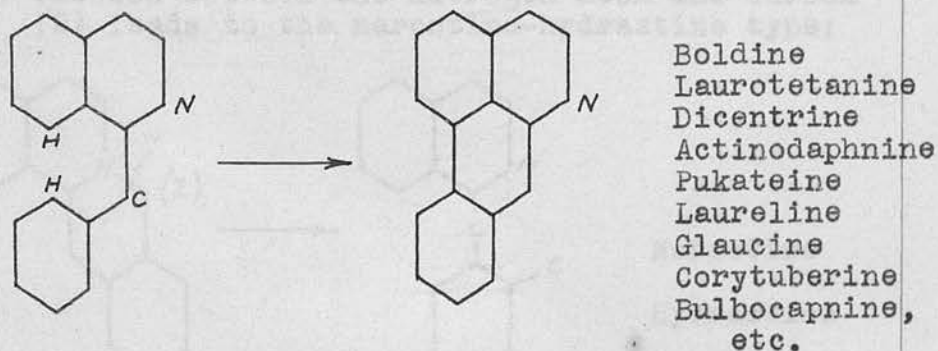
(ii) Recently a number of interesting alkaloids have been isolated whose ring systems consist of two benzyl-iso-quinolines joined together by bridge oxygen atoms. Representatives of this group of alkaloids/

alkaloids are: oxyacanthine (29), iso-chondodendrine (30), trilobine (31) and phaeanthine (32); the following ring structure is that of oxyacanthine:

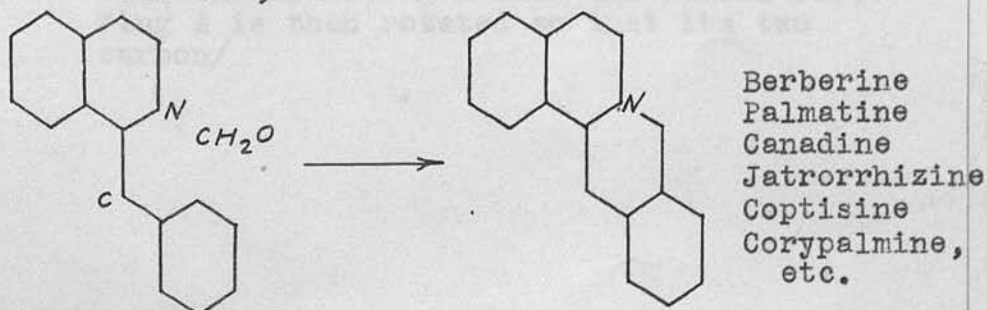


(iii) The benzyl iso-quinolines themselves can undergo two further ring closures, thus:

(a) by the loss of two H- atoms, the important group of aporphines is arrived at:

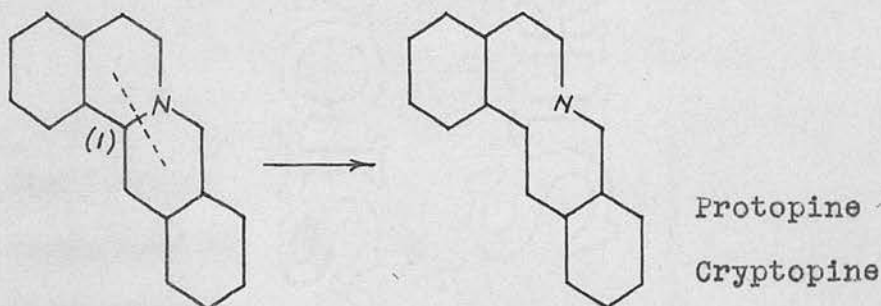


or (b) the lower ring of the benzyl iso-quinoline is rotated and by the intervention of formaldehyde the berberine structure is derived, thus:

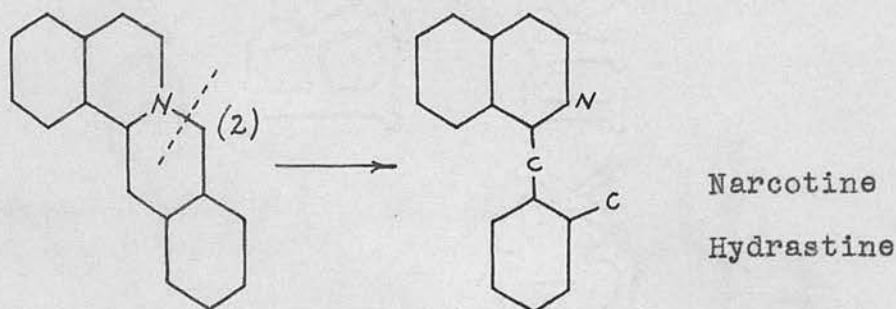


(iv) The berberine formula can, by means of the theory of Bruchhausen and Bersch (33), be made to account for three further structural types; that is to say, since the nitrogen atom is attached to three different carbon atoms, the fission of each linkage should, in turn, give rise to a new class of compounds, thus:

- (a) fission of the bond between the nitrogen atom and carbon (1) leads to the protopine type:

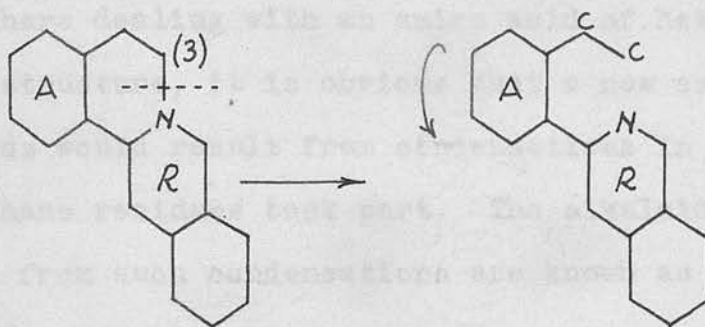


- (b) fission between the nitrogen atom and carbon (2) leads to the narcotine-hydrastine type:

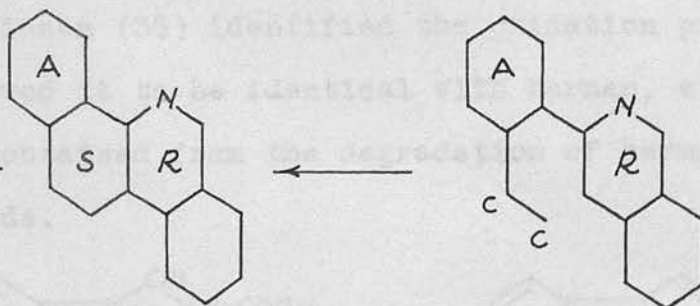


- (c) the third and most complicated of the three changes results in what is known as the chelidonine type: first, fission occurs between the nitrogen atom and carbon (3), ring A is then rotated so that its two carbon/

carbon side chain is placed in a position to attach itself to ring R, with the resulting formation of a new ring S:

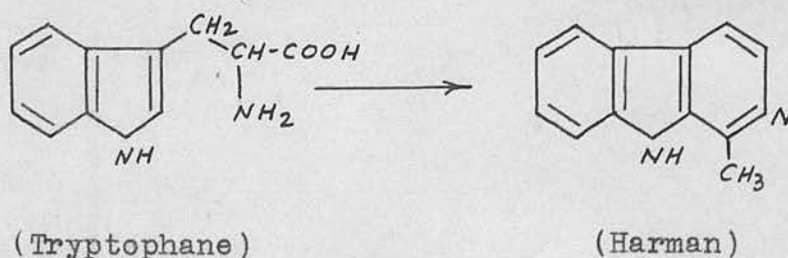


Chelidonium
Sanguinarine
Chelerythrine



Of the three typical amino acids (phenyl-alanine, tyrosine and tryptophane), mentioned above, which might serve as the precursors of alkaloids in plants, tryptophane remains to be discussed. Since we are here dealing with an amino acid of hetero-cyclic structure, it is obvious that a new series of alkaloids would result from condensations in which tryptophane residues took part. The alkaloids arising from such condensations are known as indole alkaloids.

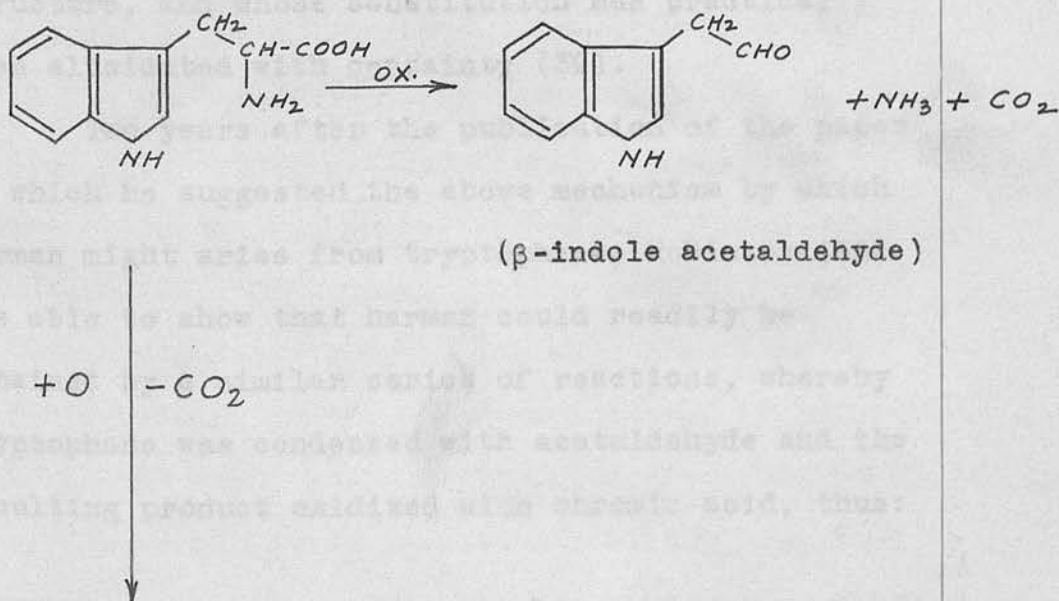
An important experiment in this connection is that due to Hopkins and Cole (34) who found that by oxidizing tryptophane with ferric chloride a substance resulted which was two carbon atoms richer than the starting material. They were unable, at first, to account for this increase in carbon-content, and it was not until several years later that Perkin and Robinson (35) identified the oxidation product and showed it to be identical with harman, a substance obtained from the degradation of harmala alkaloids.

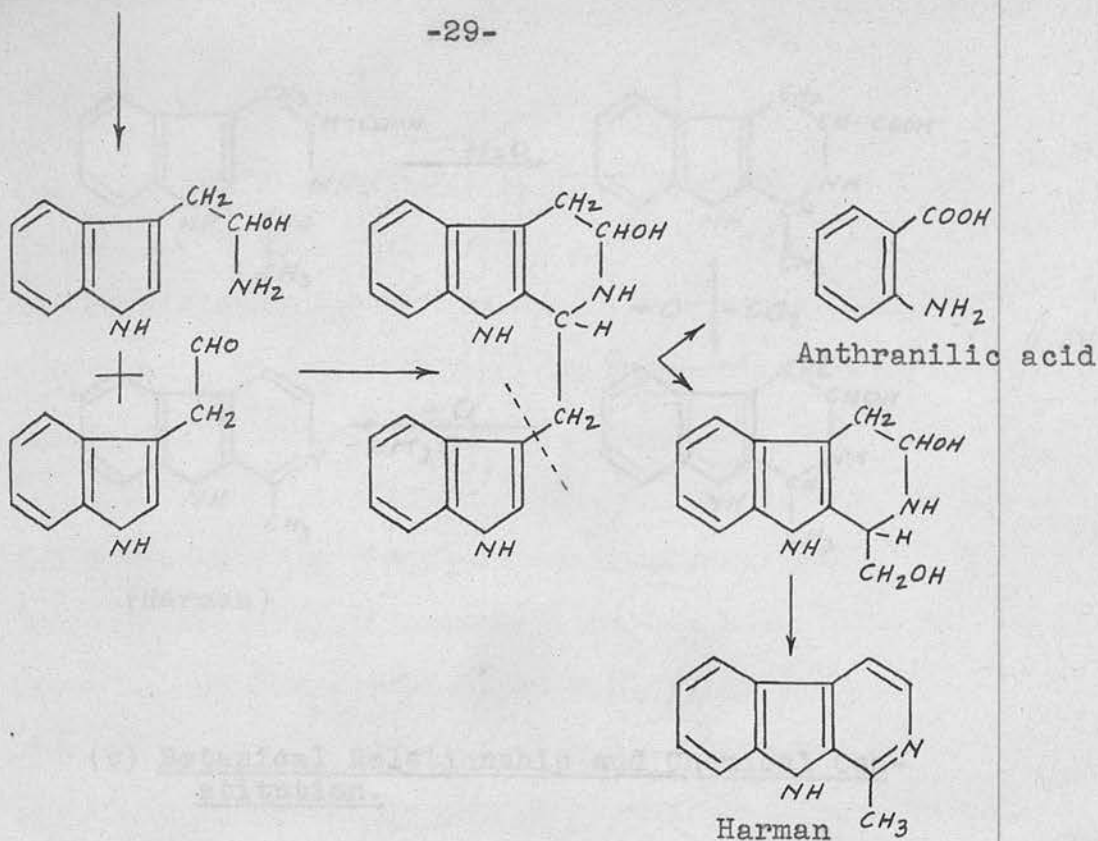


This was of far-reaching importance for two reasons, first, because it helped to clear up the constitution of a few harmala alkaloids and second, it seemed to strengthen the view that tryptophane should be considered as a precursor of some alkaloids.

To account for the increase of two carbon atoms in the above experiment, Hopkins and Cole (loc. cit.) suggested that their product may have in some way combined with either alcohol or ether - solvents used in working up the product.

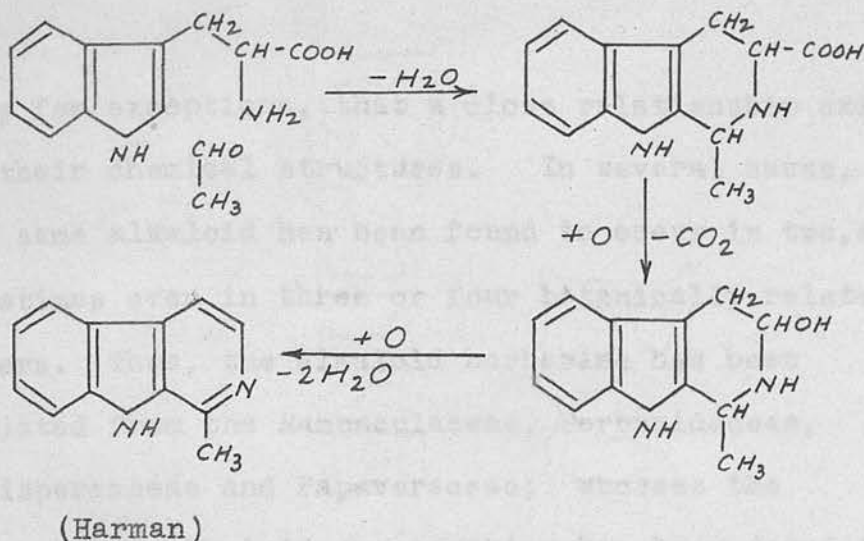
However, Robinson (35) thought this unlikely, and by applying his original views (i.e. that aldehydes may result from amines on oxidation), suggested the following series of reactions by which Harman could be derived from tryptophane:





It is interesting to note, in this connection, that oxalylanthranilic acid has been isolated as a degradation product from yohimbine (37, 38), an alkaloid which is known to contain a harman structure, and whose constitution has practically been elucidated with certainty (39).

Two years after the publication of the paper in which he suggested the above mechanism by which harman might arise from tryptophane, Robinson (36) was able to show that harman could readily be obtained by a similar series of reactions, whereby tryptophane was condensed with acetaldehyde and the resulting product oxidized with chromic acid, thus:



(c) Botanical Relationship and Chemical Constitution.

Willis, in his "Flowering Plants and Ferns" (40), has adopted the Engler system for the classification of plants according to which, botanically related natural orders are grouped into cohorts, in the same way that species are grouped into genera, and genera into natural orders.

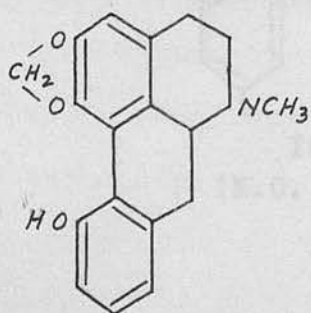
In the cohort RANALES of the Engler system, we find the following alkaloid-bearing natural orders: Ranunculaceae, Berberidaceae, Menispermaceae, Calycanthaceae, Anonaceae, Monimiaceae, Lauraceae and Hernandiaceae; whereas the very important alkaloid-bearing group, the Papaveraceae, is found to occur in the immediately following cohort RHOEADALES.

Where alkaloids have been isolated from botanically related orders, it has been found, with very/

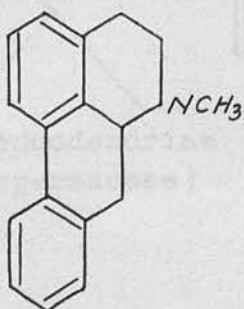
very few exceptions, that a close relationship exists in their chemical structures. In several cases, the same alkaloid has been found to occur in two, and sometimes even in three or four botanically related orders. Thus, the alkaloid berberine has been isolated from the Ranunculaceae, Berberidaceae, Menispermaceae and Papaveraceae; whereas the interesting alkaloid oxyacanthine has been found to occur in the Berberidaceae as well as in the Menispermaceae, and the alkaloid laurotetanine has been isolated from both the Lauraceae and the Hernandiaceae.

The number of cases in which the same alkaloid occurs in different natural orders is, however, small in comparison with the host of alkaloids known to-day. Nevertheless, the literature abounds in experimental evidence which bears out the existence of a close structural relationship between alkaloids originating from related natural orders.

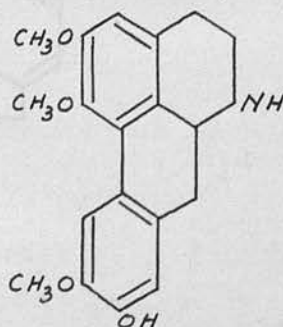
To illustrate the foregoing, we might, for example, examine those alkaloids which possess an aporphine structure, and we learn that representatives are to be found in three related natural orders, viz.



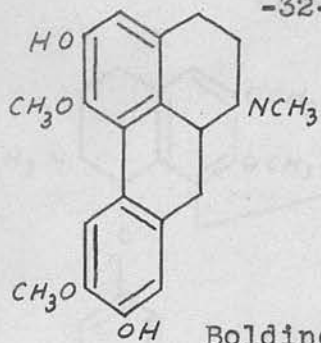
Pukateine
(N.O. Lauraceae)



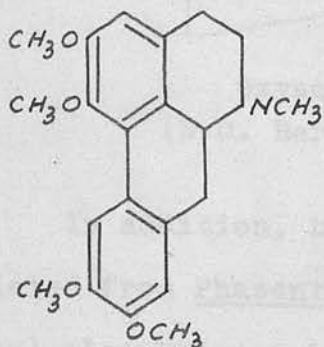
Aporphine



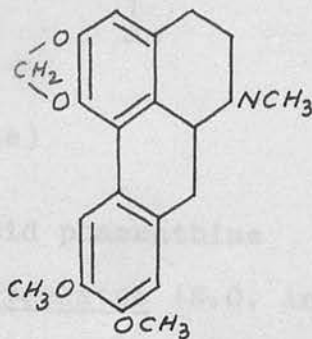
Laurotetanine
(N.O. Lauraceae)



Boldine
(N.O. Monimiaceae)

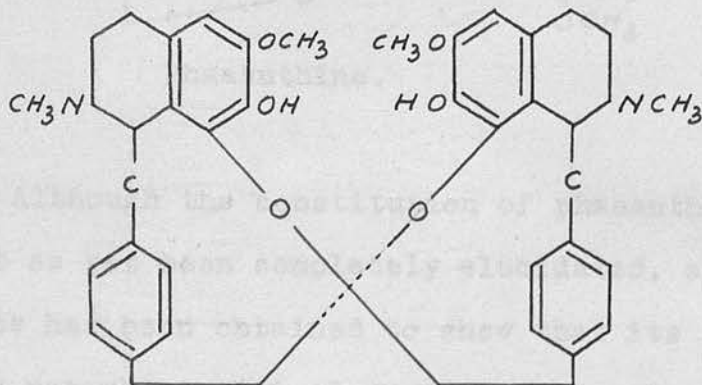


Glaucine
(N.O. Papaveraceae)

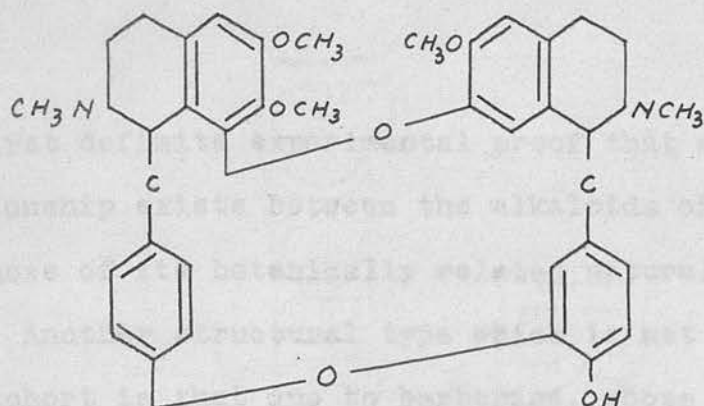


Dicentrine

The alkaloids, iso-chondodendrine (N.O. Menispermaceae) and oxycanthine (N.O. Berberidaceae) are representatives of the double molecule type (referred to in an earlier section of this thesis), and are related to each other as follows:

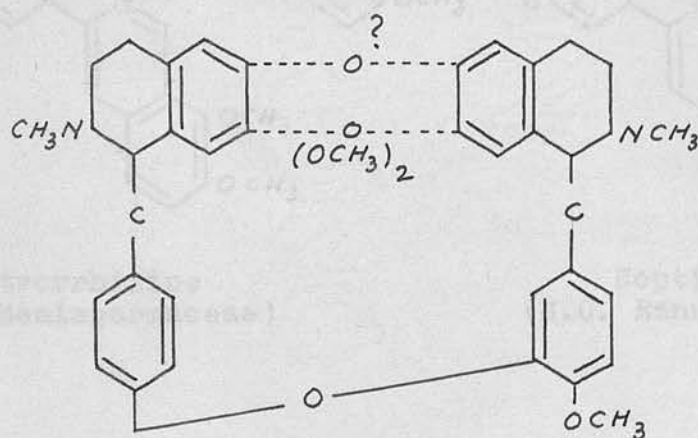


Iso-chondodendrine
(N.O. Menispermaceae)



Oxyacanthine
(N.O. Berberidaceae)

In addition, the alkaloid phaeanthine isolated from Phaeanthus ebracteolatus (N.O. Anonaceae) also belongs to this type, thus:

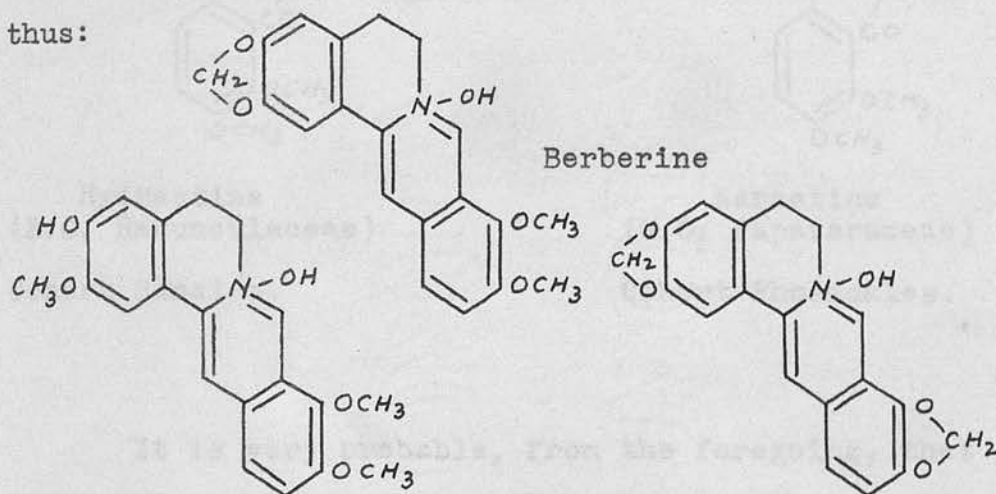


Phaeanthine.

Although the constitution of phaeanthine(32) has not as yet been completely elucidated, sufficient evidence has been obtained to show that its structure closely resembles that of oxyacanthine and of iso-chondodendrine. And it must be emphasized that this demonstration of a resemblance in structure constitutes the/

the first definite experimental proof that a chemical relationship exists between the alkaloids of Anonaceae and those of its botanically related natural orders.

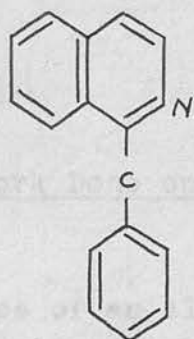
Another structural type which is met with in this cohort is that due to berberine, whose occurrence in at least four natural orders was mentioned above, thus:



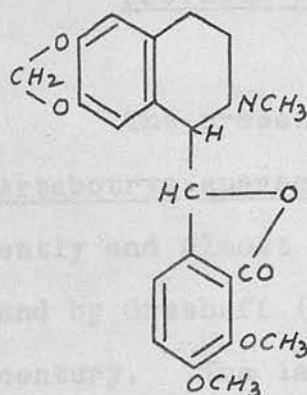
Jatrorrhizine
(N.O. Menispermaceae)

Coptisine
(N.O. Ranunculaceae)

Still another type is the papaverine or benzyl iso-quinoline structure which occurs in representatives of both the cohort RANALES and the cohort RHOEADALES, thus/

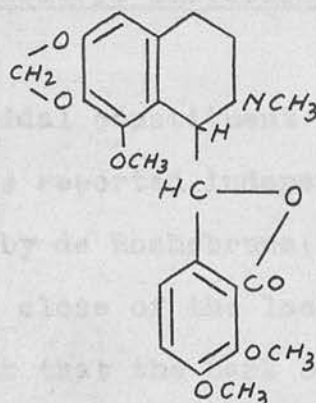


Papaverine
(Skeleton formula)



Hydrastine
(N.O. Ranunculaceae)

Cohort Ranales.



Narcotine
(N.O. Papaveraceae)

Cohort Rhoeadales.

It is very probable, from the foregoing, that other Anonaceae alkaloids would fall into one of the four main structural types illustrated above.

(d)/

(d) Theoretical.

Previous Work Done on Artabotrys suaveolens.

The presence of an alkaloidal constituent in Artabotrys suaveolens, Blume, was reported independently and almost simultaneously by de Rochebrune(15), and by Greshoff (12) towards the close of the last century. The latter pointed out that the bark of the branches, but not the leaves, contained about 0.1% of an alkaloid, accompanied by a blue fluorescent substance.

Apart from these meagre reports, nothing further appeared until Maranon (13), in 1929, re-examined a Philippine specimen of the same species and succeeded in isolating in crystalline form a small quantity of alkaloid melting at 187°, and assigned to it the name "Artabotrine"; he also proposed the empirical formula $C_{38}H_{55}O_8N$ for the substance. In addition, he prepared the crystalline hydrochloride and hydrobromide of the base and found that a few milligrams of the hydrobromide, when injected subcutaneously, were fatal to the guinea-pig.

Since Maranon (loc. cit.) had made no attempt to examine the alkaloid chemically, Santos and/

and Reyes (14) reinvestigated the problem, three years later, with this end in view. They were able to show, first of all, that Maranon's formula for artabotrine was incorrect and, from their own analyses, ascribed to it a formula $C_{21}H_{25}O_4N$. In addition they demonstrated the presence of three methoxyl groups (Zeisel), as well as one N-methyl group (Herzig-Meyer); they were unable, however, to ascertain the function of the fourth oxygen atom, the alkaloid being non-phenolic. They found artabotrine to be dextro-rotatory, $[\alpha]_D^{30} = +198.7^\circ$ (in chloroform); and besides the hydrobromide and chloroplatinate, they prepared the methiodide which served as a starting point in the Hofmann degradation which they carried out. By digesting the methiodide with freshly prepared silver chloride, and boiling the resulting methochloride with alkali, they obtained an amorphous methine-base which was neither purified nor analyzed. This was then further methylated (methyl iodide) to the methine-methiodide which, after being converted to the methine-methochloride (via silver chloride), was boiled with alkali. The trimethylamine which was split off at this point was characterized as the chloroplatinate, but the small quantity of amorphous, non-nitrogenous material/

material which resulted, was abandoned because of its "unpromising appearance".

In addition to artabotrine, Santos and Reyes (loc. cit.) claim to have isolated in small quantity, a phenolic alkaloid of melting point 182° , to which they allotted the formula $C_{20}H_{23}O_4N$. For this new alkaloid they suggested the name "Suaveoline" and demonstrated that it, too, was dextro-rotatory,

$$[\alpha]_D^{30} = + 203-206^{\circ}.$$
 By the methods of Zeisel and Herzig-Meyer, they were able to show that the alkaloid possessed three methoxyl groups and an N-methyl group respectively, and immediately concluded that the fourth oxygen atom must be due to a phenolic hydroxyl since the alkaloid was isolated from the phenolic fraction. Although plausible, their conclusion was not supported by any experimental proof whatsoever.

A Hofmann degradation also was attempted with this alkaloid using, instead of methyl iodide, methyl sulphate and alkali as the methylating agent in the intermediate stages. Here again the methine-base was amorphous; and although they detected and characterized the trimethylamine which was split off after the second methylation, the final non-nitrogenous product/

product was again abandoned due to its unpromising appearance.

Because of the ease with which the nitrogen ring was opened, Santos and Reyes (loc. cit.) believe that a tetrahydroiso-quinoline ring system exists in the alkaloid.

The above summary surveys the work done on the alkaloids of Artabotrys suaveolens, no further publications having appeared on the subject since 1932.

Present Investigation.

After the extraction of a large supply of bark from Artabotrys suaveolens, which was obtained through the kind co-operation of the Forestry authorities at Manila, several small scale experiments were carried out on the crude extracts with a view towards improving, if possible, upon the yield of alkaloid which had previously been reported. After three attempts, a method was worked out whereby the total yield of alkaloids obtained was slightly better than 0.2%, as compared with the yield of 0.1% reported by Greshoff (12).

In addition to the principal non-phenolic alkaloid artabotrine, two apparently new alkaloids were/

were isolated; one was phenolic in nature (Y) - not identical with Santos' suaveoline; whereas the other (Z) was non-phenolic in character.

Artabotrine.

The micro-analyses of the free base and also of several degradation products appeared to be in better agreement with the formula $C_{20}H_{23}O_4N$, than with the formula $C_{21}H_{25}O_4N$ recorded earlier; the C_{20} formula is therefore preferred, and is the one used throughout this work. The melting point of the base 186° agreed well with Santos' (187°); also the rotations were in agreement; our specimen $[\alpha]_D^{15^\circ} = +194.8^\circ$, as compared with $[\alpha]_D^{30^\circ} = +198.7^\circ$ (Santos).

Methoxyl and methylimino estimations revealed the presence of three O-methyl groups and one N-methyl group respectively, thus confirming previous findings. In addition, the function of the fourth oxygen atom has been determined and can now be said, with a reasonable degree of certainty, to be due to an alcoholic hydroxyl group. Moreover, this alcoholic group is unique in that it appears to be endowed with slightly acidic properties, and this latter attribute will be discussed more fully below.

Suffice/

Suffice it to say at this point that the presence of an alcoholic hydroxyl group was deduced from the following experimental evidence: although non-phenolic in nature (it gives no coloration with alcoholic ferric chloride either in hot or cold solution, nor is it appreciably soluble in boiling 10% alkali), artabotrine possesses one reactive hydrogen atom (Zerewitinoff); and on heating at 100° with acetic anhydride in the presence of a small quantity of anhydrous sodium acetate, yields a well defined mono-acetyl derivative which is slowly soluble in warm dilute mineral acid (positive Mayer reaction). Since the nitrogen atom is tertiary in character, as shown by the methylimino estimation and by the fact that artabotrine forms no nitroso-derivative, the possibility that the active hydrogen atom might be due to an $>NH$ group can be dismissed.

By the action of nascent diazomethane in absolute methyl alcoholic solution (ethereal diazomethane is apparently without action), the hydroxyl group can be methylated in practically quantitative yield when working with small quantities (0.1-0.2 gm.), and in 75-80% yield when engaged with larger ones (0.75-1.25 gm.). The methylated product, although oily and not inclined to crystallize, readily/

readily forms a well-defined methiodide which, upon analysis, clearly shows the presence of an additional methoxyl group (i.e. four in all). In chloroform, O-methyl artabotrine has a rotation of $[\alpha]_D^{16} = +182^\circ$.

The hydroxyl group in artabotrine could also be methylated by shaking a finely divided suspension of the alkaloid in 2N. aqueous sodium hydroxide with dimethyl sulphate. In addition the acid methyl sulphate residue attached itself to the nitrogen atom with the result that the product behaved like a salt, and was readily soluble in water. By adding to this solution of the "salt" in water, a concentrated aqueous solution of potassium iodide, the sparingly soluble methiodide crystallized out almost immediately.

The results of the two preceding methylation experiments appear to support the view that the hydroxyl group in artabotrine, though technically alcoholic, possesses slightly acidic properties; a true alcoholic group would have been unaffected by either diazomethane or dimethylsulphate.

It should be mentioned, in this connection, that when solutions of artabotrine in non-hydroxylic solvents (chloroform, acetone or benzene, etc.) are treated with a drop or two of ethereal ferric chloride, a turbidity develops, and a yellow to brown/

brown precipitate results. Although these observations are not exactly the same as those reported for enolic hydroxyls, for which this method of detection (41) is particularly useful, there are indications at least that the hydroxyl group in Artabotrine is unique in its behaviour.

With phenyl isocyanate in dry chloroform, artabotrine yields a clear, syrupy product (probably the phenyl carbamate) which is no longer soluble in acids. It could not be crystallized.

No methylene-dioxy group could be detected by Gaebel's test; nor could the presence of a carbonyl group be demonstrated through the oxime. Artabotrine does not possess any readily reducible double bonds; no hydrogen was absorbed when a sample was shaken with Adams' catalyst (platinum oxide) in an atmosphere of hydrogen for 6 hours - the starting material could be recovered practically quantitatively unchanged.

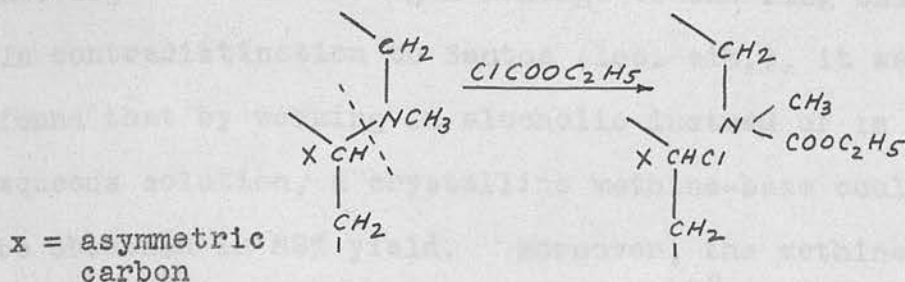
Attempts to replace the hydroxyl group in Artabotrine by halogen, with a view to the subsequent exchange of the halogen for hydrogen by means of the $(\text{Pd}-\text{CaCO}_3)$ catalyst of Busch and Stöve (42) used with such remarkable success by Harington in his work on thyroxine (43), were attended by difficulties/

difficulties. The interaction of artabotrine with thionyl chloride always seemed to result in a poly-halogenated product which was of little use for the reduction experiment.

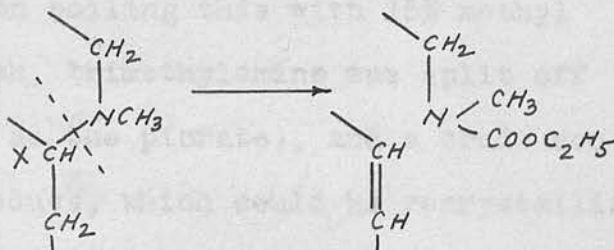
The first positive experimental evidence as to the structure of artabotrine was derived from its behaviour with ethyl chloro carbonate. When the alkaloid is treated with this reagent, a crystalline product is obtained which is neutral, optically inactive and contains no chlorine.

According to Gadamer and Knoch (44) who first studied the action of this reagent on various alkaloid structures, the formation of a neutral, optically inactive product is good evidence for the presence of a tetrahydro-iso-quinoline ring system. Moreover, they pointed out that the reagent could also be used to differentiate, with a fair degree of certainty, between the tetrahydro-papaverine type, e.g. as in laudanosine and the aporphine type of ring system, e.g. as in bulbocapnine. Thus they showed that, although the bond between the nitrogen atom and the asymmetric carbon was broken in the case of laudanosine, the chlorine atom of the reagent had attached itself to the asymmetric carbon with the result that the product was still optically active/

active:



On the other hand, the product with bulbo-capsine was optically inactive and contained no chlorine:



The specificity of this reagent for tetrahydroiso-quinoline ring systems, has been borne out by other investigators, as for example, with pukateine (45) and with boldine diethyl ether (46).

Additional evidence as to the nature of the ring system in artabotrione was obtained from a study of the Hofmann degradation which, after altering a few of the details, offered little difficulty. After twice treating with methyl iodide and boiling with alcoholic potash the nitrogen was eliminated as trimethylamine, thus showing/

showing that the nitrogen belongs to one ring only. In contradistinction to Santos (loc. cit.), it was found that by working in alcoholic instead of in aqueous solution, a crystalline methine-base could be obtained in 82% yield. Moreover, the methine base was still optically active, $[\alpha]_D^{18} = -183^\circ$ (alcohol).

Further treatment of the methine-base with methyl iodide afforded the methine-methiodide (not isolated) and on boiling this with 15% methyl alcoholic potash, trimethylamine was split off (characterized as the picrate), and a crude non-nitrogenous product, which could be recrystallized from hot ligroin, was obtained in 70-75% yield. This substance exhibited a strong, blue-violet fluorescence in solution, which is reminiscent of the blue-violet fluorescence recorded for the Hofmann degradation products of many aporphine alkaloids; moreover the substance showed slightly acidic properties, as was indicated by its solubility (rather slow) in concentrated alkali, and by the fact that upon acidification of the alkaline solution, a distinct turbidity resulted. In addition, the non-nitrogenous product also has a readily reducible unsaturated bond; and the colours which are produced/

produced when it is treated with cold concentrated sulphuric acid or with cold concentrated nitric acid are reminiscent of those observed under similar conditions with the substituted vinyl-phenanthrene obtained from the Hofmann degradation of fully methylated corytuberine (47).

Values obtained from analyses of the Hofmann degradation product derived from artabotrine agree fairly well with those required for a trimethoxy-vinyl-phenanthrol.

While contra-indicating a tetrahydro-papaverine structure, the chloroethyl carbonate experiment, mentioned above, has indicated the presence of a tetrahydro-iso-quinoline ring system; similarly the hydrogen content of artabotrine also precludes the possibility of a tetrahydro-papaverine ring system, in that this type of structure requires two hydrogen atoms more than can be accommodated by the closely agreeing duplicate analyses obtained with the alkaloid. In addition, the Hofmann degradation has shown that the nitrogen atom belongs to one ring only; this evidence would therefore dismiss the berberine type of structure in which the nitrogen atom is a member of two rings.

We have seen in a previous section of this thesis that practically all of the known alkaloids derived/

derived from Natural Orders classified in the cohort RANALES fall into four principal structural types, viz., the berberine, aporphine, papaverine and the oxyacanthine (or double molecule) type.

From the experimental evidence thus far obtained (above), the papaverine and berberine structures may be dismissed. As regards the two further possibilities, the following evidence, obtained in two ways, shows that the oxyacanthine type may also be dismissed:

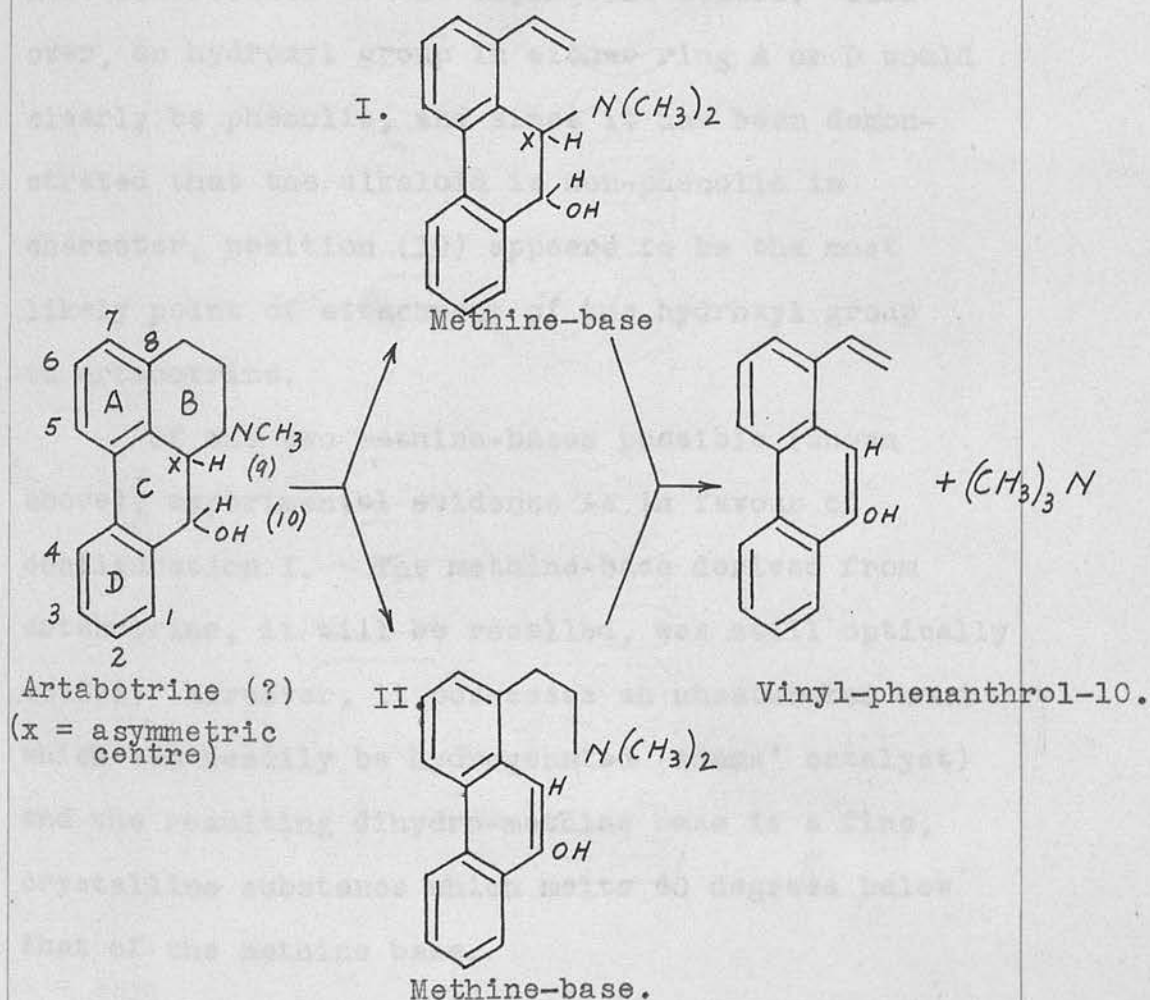
a) from X-ray analyses of artabotrine crystals, Dr E.G. Cox of the University of Birmingham, concluded that artabotrine is of the single molecule type.

b) the chemical evidence, although negative in character, is nevertheless useful. It is based on the failure to obtain any product whatsoever from an ozonization experiment carried out on the methine base. This method, it may be emphasized, was probably the most useful tool which Bruchhausen, working with oxyacanthine (49), and which Kondo, working with trilobine (31), had at their disposal, in that such characteristic products were always obtained.

From the foregoing, it seems reasonable to assume/

presence of an hydroxyl group.

The group responsible for this similarity in behaviour of the Hofmann product with 9 or 10 phenanthrol probably arises from the alcoholic hydroxyl originally present in artabotrine, and the mechanism of the Hofmann degradation may be represented as follows: (Note: the methoxyl groups are omitted here).



The/

The possibility that the hydroxyl group, in artabotrine, is attached to the asymmetric carbon atom (9) may be dismissed since, in this position it would be tertiary in character, and as such would readily be eliminated as water on boiling with potash (e.g. first step of Hofmann), and the effect would probably be even more far-reaching than the mere destruction of the asymmetric centre. Moreover, an hydroxyl group in either ring A or D would clearly be phenolic, and since it has been demonstrated that the alkaloid is non-phenolic in character, position (10) appears to be the most likely point of attachment of the hydroxyl group in artabotrine.

Of the two methine-bases possible (shown above), experimental evidence is in favour of configuration I. The methine-base derived from artabotrine, it will be recalled, was still optically active; moreover, it possesses an unsaturated bond which can readily be hydrogenated (Adams' catalyst) and the resulting dihydro-methine base is a fine, crystalline substance which melts 40 degrees below that of the methine base.

On the other hand, configuration II for the methine base can fulfil none of these conditions; it/

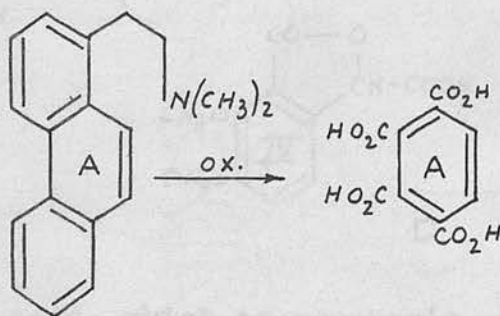


it would be optically inactive, and the double bond which has been introduced has merely served to aromatize the central ring, and in this state the substance would not be expected to absorb hydrogen under the conditions of the above experiment.

Upon examining the assumed artabotrine formula shown above, an interesting, although purely speculative, point comes to mind. Ring C may conceivably be thought of as a cyclohexadiene-ol, and as such, the hydroxyl group, although technically alcoholic, might be expected to show weakly acidic properties. This would, perhaps, account for its reaction both with nascent diazomethane and with dimethyl sulphate.

A Hofmann degradation in which O-methyl artabotrine was used as a starting point proceeded smoothly, with the exception that the vinyl compound, although obtained in excellent yield, rapidly polymerized. Oxidation attempts on this substance have afforded a small quantity of crystalline material, but this has not yet been fully investigated.

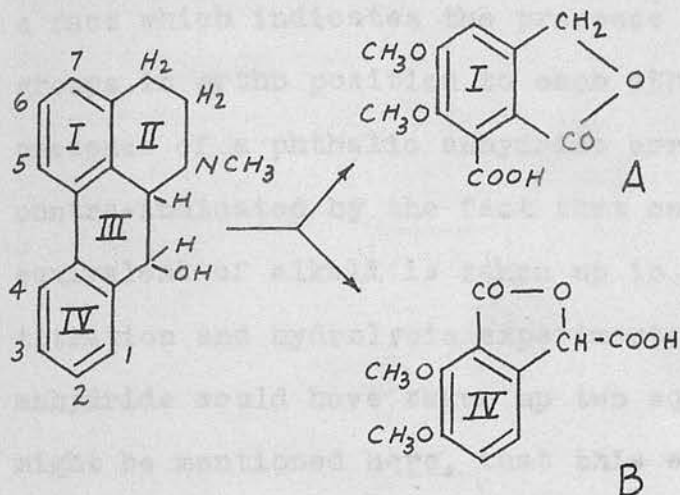
One of the most characteristic breakdowns of the aporphine alkaloids can be effected by an oxidation of the methine-base with concentrated nitric acid to 1, 2, 3, 4 benzenetetracarboxylic acid (50), when only the central ring survives:



A similar experiment carried out with artabotrine resulted in practically complete destruction of the molecule; nothing definite could be isolated. This was perhaps due to the fact that the central ring in artabotrine is, in all probability, the one which carries the hydroxyl group, and hence would be almost the first to be destroyed by oxidizing agents.

On the other hand, although the yields were poor, oxidation of artabotrine with potassium permanganate in neutral, aqueous solution, gave rise to an interesting compound - a lactone acid; the empirical formula of which permits of the two following/

following structural types. (Note: the methoxyl groups are arbitrarily placed).



Note: Inspection shows that structure A could be derived only from a ring such as I.

Similarly: Structure B could arise only from ring IV.

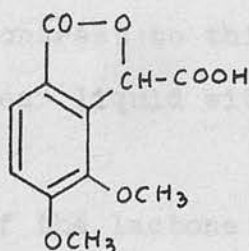
The acid, which is monobasic, possesses two methoxyl groups and has an empirical formula $C_{11}H_{10}O_6$. If a known excess of alkali is added to the titration solution after the neutralization of the free carboxyl, the solution boiled during 10 minutes, cooled and back-titrated, exactly one more equivalent of alkali is found to have been taken up; a lactone arrangement is therefore indicated. The substance sublimes in high vacuum without decomposition, indeed this is the only way in which it may be properly purified, since the acid as obtained from the experiment is tinged with a light salmon colour which cannot be removed even by three recrystallizations. When the substance is heated with resorcinol and/

and concentrated sulphuric acid in the usual way, a strong, positive fluorescein test is obtained; a fact which indicates the presence of two carbonyl groups in ortho position to each other. The presence of a phthalic anhydride arrangement is contra-indicated by the fact that only one additional equivalent of alkali is taken up in the above titration and hydrolysis experiment; phthalic anhydride would have taken up two equivalents. It might be mentioned here, that this experiment was carried out on two separate specimens: (a) the substance purified by recrystallization and (b) a sample purified by sublimation; in each case was only one equivalent of alkali taken up after first neutralizing the carboxyl group.

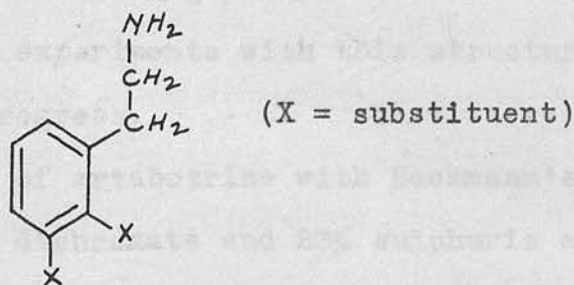
To obtain more definite proof as to which type of lactone acid we were dealing with, synthetic experiments were undertaken.

Six isomers are possible for the arrangement shown in formula B (page 54), but of these only three were known and two could be eliminated immediately on the basis of their widely divergent melting points. The third known acid was synthesized because of its promising melting point, but a mixed melting point of this with the natural acid/

acid was depressed well below that of either component. Of the three unknown isomers, two were synthesized and found not to be identical with the natural acid. A synthesis of the sixth isomer was attempted, but without success; the following formula shows the steric difficulties involved in this isomer:



Moreover, such an arrangement is also considered unlikely on biochemical grounds, since it would place the two methoxyl groups in positions (1) and (2) in the formula given above for artabotrine which, if the aporphine assumption is correct, would require for its phytochemical synthesis the following substituted phenylethylamine:



Such an arrangement of substituents is not known to exist in nature.

In/

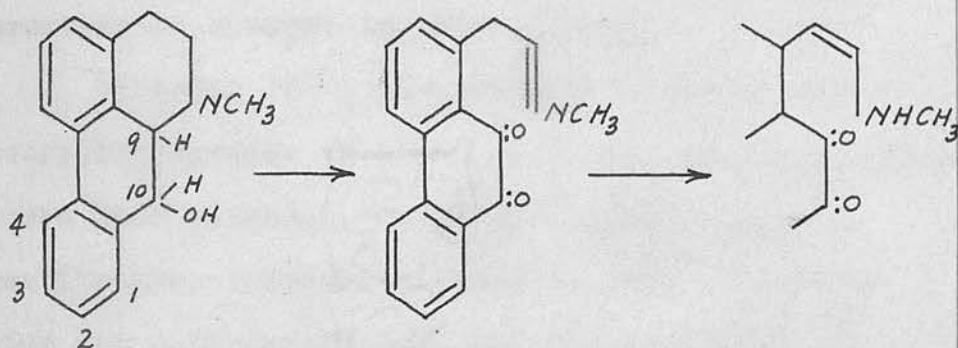
In addition, none of the synthetic acids (i.e. lactone structure as in (B) gave a positive fluorescein test. Moreover, they all appeared to split off carbon dioxide at their melting points, a phenomenon which is not at all surprising when we consider the manner in which the carboxyl group is attached. In contrast to this, the natural acid melted to a clear liquid without any signs of effervescence.

Inspection of the lactone structure as illustrated in A, shows that the fluorescein test can be accounted for by the ortho arrangement of the carbonyl groups. Moreover, the carboxyl group is here aromatically bound as, for instance, in benzoic acid, and this might well account for the fact that the natural acid can be sublimed without decomposition; also no carbon dioxide would be expected to split off at the melting point.

Synthetic experiments with this structure in view are in progress.

Oxidation of artabotrine with Beckmann's mixture (potass. dichromate and 23% sulphuric acid) gave rise to a small amount of a deep red-coloured substance which, although still basic, behaved very much like an ortho-quinone. Its deep colour was rapidly discharged by sulphur dioxide and it gave/

gave a positive Laubenheimer reaction (indicative of phenanthraquinone). A possible mechanism for this oxidation would be:



It should be mentioned, in this connection, that control experiments were carried out in exactly the same way with the aporphine alkaloids, laurotetanine (hydroxyl in position 2, above) and with pukateine (hydroxyl in position 4); in neither case was any coloration obtained, the ethereal extracts of the oxidation products being absolutely colorless.

It is conceivable that the peculiar arrangement of the hydroxyl group in artabotrine (assumed to be at position 10) accounts for the formation of a substance possessing o-quinoid properties. A rough parallel is had in the case of 10-phenanthrol which gives rise to phenanthraquinone, when oxidized with chromic acid mixture in the same way (51).

A comparison of the ultra-violet absorption spectra of artabotrine with those of known aporphine alkaloids, obtained under identical conditions, appears to confirm the view that an aporphine structure is present in artabotrine.

Girardet (52), who recently examined the absorption spectra of a number of aporphine alkaloids, showed that although the method is not strictly quantitative, comparative results can be obtained which are both trustworthy and reproducible. In addition to demonstrating that alkaloids of related structure gave the same general type of absorption band or bands, he was able to show that the replacement of such substituents as hydroxyl by methoxyl or vice-versa, has little effect on the positions of the maxima and minima of the absorption bands, or at most causes a slight shift of the main bands either to the red or to the blue end of the spectrum, depending on the nature of the incoming group. Likewise the replacement of a methylene-dioxy group by two vicinal methoxyls or vice-versa, seldom results in a shift of the bands by more than 50 Å.

The absorption spectra of laurotetanine and of dicentrine were examined for comparison purposes, but their respective spectro-photographs are included here/

here (see p. 126) because they graphically confirm Girardet's findings in the matter of structurally related alkaloids giving rise to similar absorption spectra. Except for the differences in kind, the substituents occupy the same relative positions in either alkaloid.

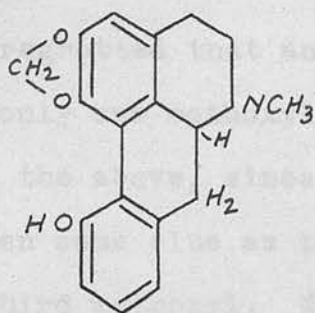
Upon inspection of the respective photographs (see p. 125), it will be seen that in the case of pukateine (an aporphine alkaloid of known structure) absorption appears to be slightly more persistent (or greater) than in that of artabotrine. On the other hand, the first absorption band of artabotrine is of slightly greater width than that of pukateine, but apart from these minor differences, it is difficult not to notice the striking resemblance which exists in the absorption spectra of the two alkaloids.

As the photographs do not show very clearly the exact values, the frequencies for the maxima and minima of the two alkaloids are given here:

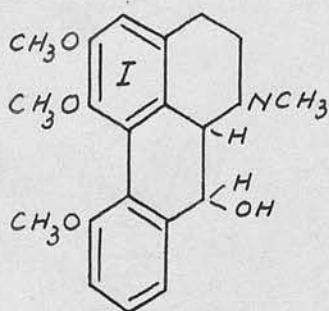
Pukateine	-	3240	3520	3745	3970
Artabotrine	-	3210	3490	3745	3990

The structure of pukateine is known to be
as/

as follows: (45, 53).

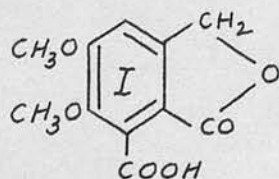


It seems reasonable to assume from the close similarity in absorption spectra and from the findings of Girardet (*loc. cit.*) in regard to substituents, that the methoxyl groups in artabotrine are situated in the same relative positions as the hydroxy and methylene-dioxy groups are in pukateine:



The effect of the hydroxyl group in artabotrine appears to be small.

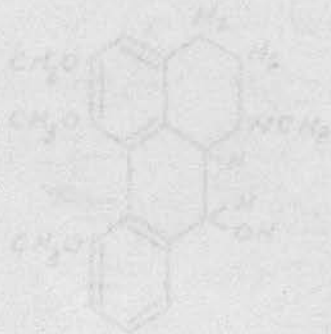
As regards ring I (in the formula immediately above), such an orientation of the methoxyl groups would support the structure suggested for the lactone acid obtained by the oxidation of artabotrine, namely,



It is to be regretted that an oxidation product possessing only one methoxyl group was not obtained along with the above, since this would have, no doubt, given some clue as to the point of attachment of the third methoxyl. No such substance resulted, even though the oxidation was repeated three times.

case, before death occurred were remarkably similar. They appear also to agree with the pharmacological observations which Parson made with his specimen of artemesine (13).

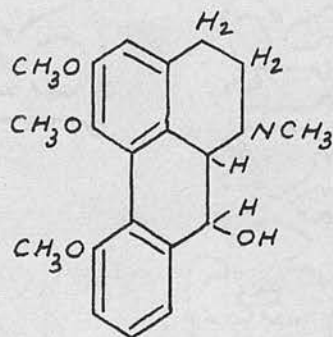
From the experimental evidence which has thus far been obtained, the author believes that the following is the most probable structure of artemesine:



The synthesis of this alkaloid, which is being undertaken by another worker in the laboratory will be the final test of the correctness of the author's views.

Through the kind co-operation of Dr M. Guggenheim of the Hoffmann-La Roche Co., (Basel), the pharmacological action of artabotrine was tested and compared with that of thebaine, an alkaloid of the morphine group. Although artabotrine was found to be roughly one-thirteenth as toxic as thebaine (mouse), the external symptoms observed in either case, before death occurred were remarkably parallel; they appear also to agree with the pharmacological observations which Maranon made with his specimen of artabotrine (13).

From the experimental evidence which has thus far been obtained, the author believes that the following is the most probable structure of artabotrine:



The synthesis of this alkaloid, which is being undertaken by another worker in the laboratory, will be the final test of the correctness of the author's views.

Phenolic alkaloid (Y).

When the phenolic fraction was first worked up, about 3 grams of a well crystallized alkaloid were obtained. This was, at first, thought to be the new phenolic alkaloid "Suaveoline", which Santos (14) reported earlier. Contrary to all expectations, this alkaloid gave no colour reaction with ferric chloride in alcoholic solution; moreover, its crystalline form closely resembled that of artabotrine and in addition, the melting points of the two substances lay only 4° apart. These facts led to the suspicion that the two alkaloids were either very closely related or might even be identical with each other. The latter was confirmed when a mixture of approximately equal parts of the two substances melted without depression. The m.p. of artabotrine is 186° (Santos 187°); after several recrystallizations the melting point of the "new" alkaloid was found to be 184-185° (Santos 182°); mixed melting point of the two, 184-185°. It is clearly evident, therefore, that Santos' "suaveoline" was nothing more than impure artabotrine; his rotations for the two alkaloids, it will be remembered, were: artabotrine $[\alpha]_D^{30} = +198.7^\circ$ and that of suaveoline $[\alpha]_D^{30} = +202-206^\circ$, values which might well represent the experimental/

experimental limits for one and the same substance. It is to be regretted that he neglected to take a mixed melting point of the two substances.

Apart from the foregoing, the fact that artabotrine, an apparently non-phenolic alkaloid, is found to occur in a phenolic fraction is both interesting and puzzling. The purified base derived from the phenolic fraction was, as far as could be seen, insoluble even in warm alkali and, as was mentioned above, gave no colour reaction with alcoholic ferric chloride.

An explanation for this anomalous behaviour of artabotrine may, perhaps, be forthcoming from an examination of past experimental evidence. We have seen that artabotrine possesses an hydroxyl group which is peculiarly endowed with slight acidic properties (as shown by its reaction with such methylating agents as diazomethane and dimethyl sulphate). It is conceivable, therefore, that during the separation of phenolic from non-phenolic alkaloids, which was accomplished by repeated extraction with 4% alkali of the ether solution containing total alkaloids, a small amount of artabotrine was carried over into the phenolic fraction.

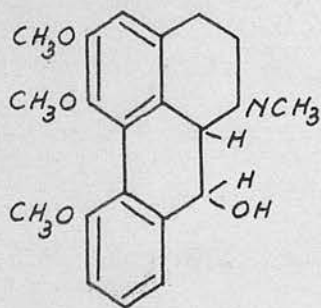
The concentrated mother liquors from the phenolic/

phenolic fraction, after standing for a week, deposited a very small amount of crystalline material. Unlike artabotrine, which crystallizes in the form of stout prisms, this substance was present in the form of fine, silky needles. After two recrystallizations from ethyl alcohol they showed a melting point of 232° . When mixed with an approximately equal quantity of artabotrine (186°) the mixture melted between $174-175^{\circ}$, with softening at 170° . In contrast to artabotrine and to the first substance isolated from the phenolic fraction, this new alkaloid was freely soluble in cold 2N. sodium hydroxide, and with alcoholic ferric chloride a deep purple coloration formed instantaneously.

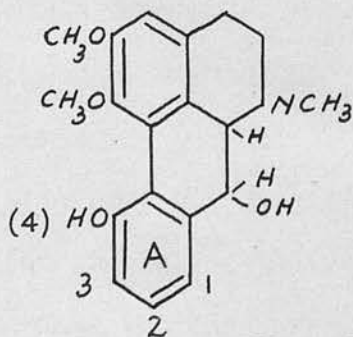
Analysis of the purified material gave rise to the empirical formula $C_{19}H_{21}O_4N$, and its rotation was found to be $[\alpha]_D^{15^{\circ}} = +164^{\circ}$ (in chloroform); compare artabotrine, $C_{20}H_{23}O_4N$, $[\alpha]_D^{15^{\circ}} = +194.8^{\circ}$ (chloroform). The alkaloid possesses two methoxyl groups (Zeisel) and one N-methyl group (Herzig-Meyer); in addition it shows the presence of two active hydrogen atoms (Zerewitinoff). Since this alkaloid is tertiary in character ($=NCH_3$), and clearly/

clearly phenolic, at least one active hydrogen must be due to a phenolic hydroxyl, the other may be due to an alcoholic group as in artabotrine. There are indications that such is the case since, like artabotrine, this phenolic alkaloid can be completely methylated (nascent diazomethane) to an oily base which, analyzed through its readily formed, crystalline methiodide, clearly shows the presence of four methoxyl groups as compared with two in the original substance. Although the melting point of its methiodide is roughly 7° below that of the methiodide of O-methyl artabotrine (246° as compared with 253°), the mixed melting point of the two was found to be $248-249^{\circ}$, so that no actual depression occurred.

Admittedly the evidence is very scant; despite this however, it seems plausible to assume the existence of a close relationship between the two alkaloids, perhaps as follows:



Artabotrine
(?)



Phenolic
alkaloid(?)

The phenolic hydroxyl is placed in position (4) in ring A, for the following reason. The alkaloid gives a positive Pellagri reaction which, according to Gadamer (54) occurs only with those phenolic alkaloids in which the para position to the hydroxyl group is unoccupied. Inspection of the formula given above for the phenolic alkaloid shows that only in ring A do two positions exist which are para to each other, viz., 1 and 4. Position 1, as has been shown in the case of artabotriline, is unlikely to be substituted on biochemical grounds; position 4 may therefore be assumed as the point of attachment of the phenolic hydroxyl group.

It is proposed to retain the name "suaveoline" for this new phenolic alkaloid.

For distinction to the latter part of which are crystalline, the new base, liberated from the hydrochloride, was a yellow, waxy-like substance which could not be crystallized. The hydrochloride, on the other hand, could readily be purified, and from an analysis of this salt, the following empirical formula was indicated:

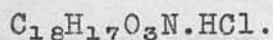


The rotation of the free base, in chloroform, was found to be $[\alpha]_D^{25} = -10.0^\circ$; that of the hydrochloride

Non-phenolic alkaloid (Z).

During the process of separating the crude bases from neutral, non-alkaloidal matter (i.e. by extracting the alkaloids from ether solution with dilute hydrochloric acid), a small quantity of fine crystalline material separated out of the acid solutions. At first, this was thought to be the hydrochloride of artabotrine and as such was filtered off and set aside. The filtrate was allowed to stand for a few days, but deposited nothing further during this interval.

Upon closer examination of the above crystalline material, however, it was found to be the hydrochloride of a new alkaloid which differed radically from both artabotrine and suaveoline. In contra-distinction to the latter two (both of which are crystalline), the new base, liberated from its hydrochloride, was a yellow, varnish-like substance which could not be crystallized. The hydrochloride, on the other hand, could readily be purified, and from an analysis of this salt, the following empirical formula was indicated:



The rotation of the free base, in chloroform, was found to be $[\alpha]_{\text{D}}^{17^\circ} = -18.9^\circ$; that of the hydrochloride, in/

in absolute alcohol, $[\alpha]_D^{17} = -41.8^\circ (\pm 4.18^\circ)$.

The hydrochloride is very sparingly soluble; 200 parts of warm water (50-60°) being required for the solution of 1 part of the salt.

Unlike artabotrine or suaveoline, both of which are tertiary bases ($=NCH_3$), this new alkaloid, for which the name "Artabotrinine" is suggested, is secondary in character ($=NH$). It readily forms a crystalline nitroso derivative which responds to the Liebermann reaction. Artabotrinine is also different in several other ways, viz., it possesses a methylene-dioxy group and but one methoxyl. Together these two groups account for the three oxygen atoms which the empirical formula requires. No N-methyl group could be detected by the method of Herzig-Meyer. Moreover, artabotrinine also possesses one active hydrogen atom (Zerewininoff) which, since the base is secondary in nature, must be due to the imino group ($=NH$). The alcoholic hydroxyl group present in the two previous alkaloids is here conspicuous by its absence.

The imino group in artabotrinine can be methylated to the corresponding N-methyl compound by/

by means of formaldehyde and formic acid (55, 56). The resulting product (obtained in 50-60% yield) is nicely crystalline and melts at 132-133°. N-methyl artabotrinine is isomeric with O-methyl pukateine, an aporphine alkaloid of known constitution whose melting point (137°) is very close to that of the former. They are not, however, identical; a mixture of approximately equal parts of the two substances melted between 98-99°; moreover, their rotations differ widely. The rotation of N-methyl artabotrinine is $[\alpha]_D^{16} = -53.8^\circ$ (in absolute alcohol), whereas that of O-methyl pukateine is recorded as $[\alpha]_D^{15} = -261^\circ$ (in absolute alcohol) (45).

In contrast to artabotrinine itself, which does not form a methiodide under ordinary conditions (i.e. refluxing with methyl iodide in methyl alcoholic solution), N-methyl artabotrinine readily forms a crystalline methiodide in the cold.

It was found that the methylene-dioxy group in artabotrinine could be hydrolysed with 40% sulphuric acid and phloroglucinol, a method worked out by Spaeth and Quietensky (57), and subsequently employed with success by Spaeth and co-workers (58). The resulting dihydroxy compound, although amorphous, behaved normally in that it gave a deep blue-green coloration/

coloration with alcoholic ferric chloride (catechol type), and easily reduced Fehling's solution. However, due to the bad yield in which it was obtained, it was methylated without previous purification.

The dihydroxy compound reacts violently with nascent diazomethane in absolute methyl alcoholic solution; it should be diluted. The methylated product is an oil which is not inclined to crystallize. Unlike artabotrinine hydrochloride, the hydrochloride of its corresponding dimethoxy compound is relatively soluble, and no definite salt could be obtained with the small quantity of material at hand.

A methoxyl estimation carried out on the oil, though it betrayed an impure substance, indicated that the methylated product possessed three methoxyl groups in contrast to the lone methoxyl present in artabotrinine.

A similar experiment employing N-methyl artabotrinine would probably lead to better results, in that the tertiary character of the nitrogen atom would, no doubt, permit of a methiodide readily being formed, and this could be purified.

The absorption spectra of artabotrinine-hydrochloride was examined at the same time that the absorption/

absorption spectra of artabotrine and of the comparative aporphine alkaloids were studied; the result is shown in the accompanying photograph (see p.127).

Although by no means as striking as in the case of artabotrine or pukateine, there appears to be a slight resemblance between the absorption spectra of artabotrine hydrochloride and that of dicentrine, an aporphine of known structure.

We have already seen that N-methyl artabotrine is not identical with its isomer O-methyl pukateine. This is probably due to a difference in orientation of the substituents, and this view appears to be supported by the absorption spectra obtained.

(e) Experimental.

Extraction of total alkaloids.

Through the kind co-operation of Messrs Duncan, Flockhart and Co., Edinburgh, 25 kg. of air-dried bark were ground and then percolated with 95% alcohol. The alcohol was removed under reduced pressure, and the thick, tarry extract that remained represented about 17% by weight of the bark used.

After three small scale experiments, the following method was adopted for working up the bulk of the crude extracts:

The material was ground in a mortar with silver sand and saturated aqueous sodium carbonate, and the finely divided suspension so obtained thoroughly extracted with ether. In order to remove neutral, non-basic matter, the ether solution was repeatedly shaken with small portions of dilute (1 N) hydrochloric acid until the acid extracts no longer gave a positive Mayer reaction. At this point, the acid solution slowly deposited a fine crystalline precipitate. After standing overnight, the precipitate (A) was filtered off (3 gm. = 0.012%), and the filtrate allowed to stand for 2-3 days longer; nothing/

nothing further was deposited during this interval. The filtrate was then basified with concentrated ammonia and extracted with ether. After a second passage through acid, the second ethereal solution was shaken with 4% sodium hydroxide to remove phenolic alkaloids (solution A).

The ethereal solution containing the non-phenolic fraction was again exhausted with dilute (1 N) hydrochloric acid, and the combined acid extracts transferred to a large separatory funnel, covered with ether and basified with concentrated ammonia. Owing to the insufficient amount of ether taken, a large quantity of crude, crystalline alkaloid separated out at this point; it was filtered off and dried. The ethereal solution after being dried over anhydrous sodium sulphate and concentrated on the water bath, yielded a further quantity of crude alkaloid. The combined yield of this material, which had all the properties of the non-phenolic alkaloid artabotrine, was 43-44 gm. (= 0.18%).

(1) /

(i) Artabotrine.

The alkaloid crystallizes from acetone in flat, rhomb-shaped crystals having beveled edges, also in stout prisms; after three recrystallizations from this solvent the melting point was 185-186°.

The base is readily soluble in chloroform; moderately in methyl and ethyl alcohols, acetone and benzene; sparingly in ether and in ethyl acetate; it is insoluble in petroleum ether (80-100°). In ethyl alcoholic solution no colour is produced with ferric chloride, even on warming. However, when solutions of the base in non-hydroxylic solvents (e.g. chloroform, acetone or benzene) are treated with 1-2 drops of ethereal ferric chloride, a turbidity forms and a yellow to brown precipitate results in a few moments.

Analysis of artabotrine:

Found: C, 70.55, 70.77; H, 6.98, 6.97; N, 4.11, 4.23; OCH_3 , 26.86; $\text{N}(\text{CH}_3)$, 3.63.

$\text{C}_{16}\text{H}_{10}(\text{OH})\text{N}(\text{CH}_3)(\text{OCH}_3)_3$ requires C, 70.38; H, 6.80; N, 4.11; OCH_3 , 27.29; $\text{N}(\text{CH}_3)$, 4.39.

Zerewitinoff estimations gave 1.05 and 0.97 equivalents of reactive hydrogen; one hydroxyl group/

group requires 1.00 equivalent of reactive hydrogen.

In chloroform the rotation of the alkaloid was found to be $[\alpha]_D^{15} = +194.8$ ($C = 1.86$, $l = 1$ dm); compare Santos' rotation for artabotrine $[\alpha]_D^{30} = +198.7^\circ$ (chloroform) (14).

1. Neutralisation equivalent of artabotrine.

0.2086 gm. of the base dissolved in ethyl alcohol required 6.05 c.c. of 0.104 N hydrochloric acid (methyl red as indicator); equivalent found: 331; $C_{26}H_{23}O_4N$ requires 341.

The solution was diluted slightly with water, a small quantity of Merck's charcoal added and the whole warmed on the water bath for 10-15 minutes. The charcoal was filtered off and washed free of hydrochloride (Mayer's test) with small portions of hot water, the washings being added to the main filtrate. Evaporation of this yielded the crude hydrochloride which, after two recrystallizations from methyl alcohol, was obtained as stout, colorless needles melting at $226-227^\circ$ with decomposition.

2. /

2. Methylene-dioxy group.

Artabotrine (20 mg.) and phloroglucinol (60 mg.) were heated with 40% sulphuric acid (5 c.c.) in a boiling water bath. Simultaneously tests with puketeine, laureline, laurotetanine and a blank were carried out in the same vessel. After approximately one hour, the test tubes containing puketeine and laureline showed a bulky red-orange precipitate, whereas artabotrine, laurotetanine and the blank showed merely a dirty yellow coloration. Artabotrine therefore does not contain this group.

3. Test for a carbonyl group.

Artabotrine (0.2 gm.) dissolved in a few c.c. dilute (1 N) hydrochloric acid was treated with a solution of hydroxylamine hydrochloride (0.2 gm.) and sodium acetate (0.5 gm.) in water (5 c.c.), and after being warmed on the water bath for half an hour the solution was allowed to stand for 3 days. Practically the whole of the original material was recovered unchanged (identified by mixed melting point).

4. Test for unsaturation.

Artabotrine in chloroform does not decolorize bromine, nor does it reduce permanganate in cold, rectified/

rectified acetone. No hydrogen was absorbed when a sample was shaken in an atmosphere of hydrogen (Adams' catalyst) for 6 hours.

5. Action of phenyl isocyanate.

Artabotrine (0.1 gm.) in dry chloroform (5 c.c.) was treated with phenyl isocyanate (0.1 gm.). Although the characteristic odour of the reagent was no longer perceptible after 15 minutes, the mixture was set aside for 2 days. After this interval, a small quantity of diphenyl urea was filtered off and the chloroform solution washed with small portions of dilute (1 N) hydrochloric acid to remove unchanged alkaloid. After being washed with water, the chloroform solution was dried over anhydrous sodium sulphate and evaporated, leaving behind a small quantity of a practically colorless, neutral, viscous oil which could not be crystallized. The acid washings, on being basified and worked up, yielded 45% of unchanged, original alkaloid.

6. Acetylation of artabotrine.

Artabotrine (0.25 gm.) was heated at 100° for one and a half hours with acetic anhydride (2.5 c.c.) and fused sodium acetate (0.25 gm.). The reaction mixture was cooled and the excess acetic anhydride/

anhydride decomposed with water (10 c.c.); simultaneously, a light amber coloured oil separated out which, after two hours, crystallized to a mass of fine needles. Recrystallized from slightly diluted ethyl alcohol they melted between 97-99°. Analysis of the substance revealed 2 moles of water of crystallization; the anhydrous derivative melted at 118-119°.

Found: C, 63.03; H, 6.68; N, 2.95; CH_3CO , 12.14; H_2O , 7.53.

$\text{C}_{20}\text{H}_{22}\text{O}_4\text{N}(\text{CH}_3\text{CO}) \cdot 2\text{H}_2\text{O}$ requires: C, 63.01; H, 6.92; N, 3.34; CH_3CO , 10.26; H_2O , 8.59.

Though the acetyl derivative was slowly soluble in warm, dilute (1.5 N) hydrochloric acid (positive Mayer reaction), a methiodide could not readily be formed, indicating that acetylation had probably taken place at the nitrogen atom.

Basification of the acetic acid mother liquor and extraction with ether yielded a very small quantity of amorphous material which was still acid soluble and gave a positive Mayer test. It could not be crystallized.

7. Action of nascent diazomethane.

A solution of artabotrine (0.1 gm.) in 20 c.c. absolute methyl alcohol was treated at 0° with 3 c.c. nitroso N-methyl urethane. Ten drops of methyl alcoholic potash (25%) were added and the whole set aside in the refrigerator for 18 hours. After this interval, another 0.75 c.c. of reagent was added followed by 7 drops of alcoholic potash, and the solution again left overnight in the refrigerator.

The solution was diluted with 20 c.c. of slightly acidified water to decompose the excess diazomethane, and the alcohol removed on the water bath under reduced pressure. The aqueous solution which remained was basified with ammonia and extracted with ether. After drying over anhydrous sodium sulphate the ether was evaporated leaving behind 90 mg. of a practically colourless, viscous syrup.

A similar experiment carried out with 2.4 gm. artabotrine and corresponding quantities of reagents, yielded 1.8 gm. O-methyl artabotrine. Unlike the parent substance artabotrine, the methylated product is readily soluble in both ether and alcohol.

In chloroform, O-methyl artabotrine shows a rotation/

rotation of $[\alpha]_D^{16} = +182.2^\circ$ ($c = 2.9$; $l = 2$ dm).

Since the syrup could not be induced to crystallize, even by seeding with a crystal of artabotrine, about 0.1 gm. was taken up in a few c.c. of absolute methyl alcohol and refluxed on the water bath for thirty minutes with 0.5 c.c. methyl iodide, and the solution allowed to stand at room temperature over-night. The methiodide was precipitated by the addition of dry ether, and after two recrystallizations from methyl alcohol was obtained in the form of fine, colourless needles of m.p. $254-255^\circ$ with decomposition.

Found: OCH_3 , 26.10%

$\text{C}_{18}\text{H}_{18}\text{N}(\text{OCH}_3)_4\text{I}$ requires 24.95.

8a. Action of dimethyl sulphate.

Finely divided artabotrine (0.6 gm.) was suspended in 15 c.c. of 2 N. sodium hydroxide, treated with 1 c.c. of dimethyl sulphate and the whole shaken for 30 minutes. After this interval another 1 c.c. portion of dimethyl sulphate was added followed by further small quantities of sodium hydroxide and the suspension was again shaken for 15 minutes.

The/

The reaction mixture was then heated for 2 hours on the water bath with 30 c.c. of 17% sodium hydroxide. The cooled solution was extracted with chloroform, the extract dried over anhydrous sodium sulphate and on concentrating the solution to small volume, a fine, crystalline precipitate slowly settled out. Filtered and dried, the yield was 0.6 gm. After two recrystallizations from methyl alcohol-ether, the product was obtained in the form of tiny, blade-shaped needles which melted at 255-256° with decomposition.

The substance is insoluble in ether, sparingly in acetone, and readily soluble in methyl alcohol and in chloroform. It is, moreover, soluble in water, and this fact together with its rather high melting point, appears to indicate that the nitrogen atom has entered into salt formation, probably with an acid methyl sulphate residue; a view which is supported by the presence of sulphur in the molecule. The nitrogen atom as well as the hydroxyl group of the original alkaloid appears to have been methylated. Found: C, 57.41; H, 6.24; N, 2.94; S, 6.83; OCH_3 , 32.23.

$\text{C}_{18}\text{H}_{16}\text{O}_3\text{NS}(\text{OCH}_3)_5$ requires: C, 57.38; H, 6.44; N, 2.91; S, 6.64; OCH_3 , 32.22.

8b. Formation of methiodide of above product.

To a solution of 30 mg. of the above substance in water (1 c.c.), a solution of potassium iodide (20 mg.) in water (0.5 c.c.) was added. After several minutes, the sparingly soluble methiodide separated out. It was redissolved by the addition of another 1 c.c. of water and warming the solution. The methiodide crystallized out, on slow cooling, in the form of clusters of stout prisms which were filtered and dried. M.p. 252-253° with decomposition.

Found: I, 24.0%.

$C_{22}H_{28}O_4NI$ requires: I, 25.5%.

When this methiodide was mixed with an approximately equal quantity of the methiodide of O-methyl artabotrine obtained via the diazomethane method, the melting point was not depressed.

9. Action of chlorethyl carbonate.

To a solution of 0.5 gm. (1 mol) artabotrine in chloroform (15 c.c.) to which several small chips of ice had been added, 0.32 gm. (2 mols) chlorethyl carbonate and 0.28 gm. (3.5 mols) solid potassium hydroxide were added and the mixture shaken constantly for/

for 1 hour. After this interval, more ice followed by another 0.32 gm. (2 mols) chlorethyl carbonate and 0.28 gm. (3.5 mols) potassium hydroxide were added and the mixture again shaken for 1 hour. The chloroform layer was drawn off, washed several times with dilute (1 N.) hydrochloric acid and dried over sodium sulphate. (The acid washings gave no Mayer reaction, thus indicating that the nitrogen atom of the alkaloid had been acted upon). Evaporation of the chloroform yielded a neutral, amber-coloured, viscous syrup which solidified when rubbed with dry ether. Because of its sparing solubility in ether, it could be recrystallized from this solvent, and after two recrystallizations the substance was obtained in the form of slightly orange-coloured rhomb-shaped plates of melting point 109-110°.

Found: C, 66.94; H, 6.63.

$C_{20}H_{22}O_4N(COOC_2H_5)$ requires: C, 66.84; H, 6.53.

A solution of 0.5789 gm. of this substance in 20 c.c. chloroform at 15° had no effect upon the plane of polarized light when examined in a 1 dm. tube. A tetrahydro-isoquinoline ring system is thus indicated (44).

10. Methiodide of artabotrine.

Artabotrine (1.7 gm.) in absolute methyl alcohol (25 c.c.) was warmed under reflux for 15 minutes with 3.2 c.c. methyl iodide, and the cooled solution allowed to stand over-night. The crystalline methiodide which separated out was filtered and dried; yield 2.4 gm. (quantitative). After two recrystallizations from methyl alcohol, fine, colourless needles were obtained melting at 224-225° with decomposition.

Found: C, 52.16; H, 5.47; N, 2.72; I, 27.57.

$C_{26}H_{23}O_4N.CH_3I$ requires: C, 52.17; H, 5.38; N, 2.89; I, 26.29.

11a. Methine base of artabotrine.

Artabotrine methiodide (2.4 gm.) was boiled under reflux for five and a half hours with 120 c.c. ethyl-alcoholic potash (20%). The cooled solution was diluted with 200 c.c. water and thoroughly extracted with ether. The combined ethereal extracts were well washed with water, dried over sodium sulphate and evaporated, leaving behind 1.45 gm. (81%) of crude, crystalline methine-base. In ether solution, the substance shows a distinct blue-violet/

violet fluorescence. After two recrystallizations from slightly diluted ethyl alcohol, the product was obtained in the form of lustrous, rhomb-shaped plates of melting point 122-123°.

Found: C, 70.73; H, 6.87, N, 4.17.

$C_{21}H_{25}O_4N$ requires: C, 70.98; H, 7.04; N, 3.93.

The methine-base is still optically active and in absolute ethyl alcohol shows a rotation of $[\alpha]_D^{18} = -183^\circ$ ($C = 1.65$; $l = 1$ dm). It also possesses a readily reducible unsaturated bond since, when a solution of bromine in chloroform is added dropwise to one of the base in the same solvent, the bromine is decolorized instantaneously; moreover potassium permanganate is also slowly reduced in cold, rectified acetone.

11b. Catalytic reduction of methine-base.

When 140 mg. of substance in 15 c.c. absolute ethyl alcohol were shaken in an atmosphere of hydrogen with 54 mg. platinum oxide, one mole of hydrogen was absorbed within 5 minutes. After filtration, water (35 c.c.) and dilute hydrochloric acid (5 c.c. of 0.1 N) were added and the alcohol removed/

removed on the water bath under reduced pressure. The aqueous residue was basified with 2 N. sodium hydroxide and thoroughly extracted with ether; the combined extracts dried and evaporated yielding 107 mg. of a very viscous syrup. This was taken up in 5 c.c. methyl alcohol and the solution concentrated to half its volume. Water was added dropwise until a turbidity formed, and on standing over-night, the dihydro-methine base crystallized in tiny, colorless, truncated prisms of melting point 80-81°.

Found: C, 70.51; H, 7.50.

$C_{21}H_{27}O_4N$ requires: C, 70.58; H, 7.56.

The reduced product no longer decolorized bromine in chloroform.

11c. When a small quantity (0.2 gm.) of methine-base was ozonized according to the method of Bruchhausen and Gericke (49), practically the whole of the molecule was destroyed; nothing definite could be recovered.

11d. Similarly, oxidation with concentrated nitric acid after the method of Warnat (50), led to complete destruction of the molecule.

12. Hofmann degradation.

Neither the Hofmann nor Emde degradations could be made to yield a definite product when they were carried out in aqueous solution with the methine-methochloride (derived from the methine-methiodide via silver chloride). Although trimethylamine was copiously evolved and characterized (as the picrate) in either case, the yield of non-nitrogenous product was poor.

It was subsequently found, however, that better results could be obtained by working directly with the methine-methiodide in methyl alcoholic solution as follows:

The methine-base (1.45 gm.) in methyl alcohol (20 c.c.) was refluxed on the water bath for one and a half hours with methyl iodide (2.5 c.c.) and, after cooling, the solution was allowed to stand over-night. After boiling off the excess methyl iodide, methyl alcohol (90 c.c.) and solid caustic potash (15 gm.) were added and the solution boiled under reflux for five and a half hours; the evolved trimethylamine being characterized as the picrate. To the cooled solution was added 350 c.c. of water (containing sulphuric acid in slight excess over that required to neutralize the potassium hydroxide taken/

taken). A precipitation of potassium sulphate occurred, accompanied by the lighter, more flocculent vinyl compound. The suspension was thoroughly extracted with ether and the ethereal extracts, which possessed a deep red-orange colour and exhibited a strong, blue-violet fluorescence, well washed with water. After being rapidly dried over sodium sulphate the ether was evaporated, yielding 0.85 gm. (75%) of crude, brick-coloured vinyl compound.

The crude material melted at 108-109°, but it could be recrystallized (with some loss of material) from boiling ligroin (100-120°) and obtained as clusters of stout, yellow-orange prisms of melting point 115-116°.

Found: C, 72.91; H, 6.02; OCH_3 , 29.7.

$\text{C}_{16}\text{H}_{18}\text{O}(\text{OCH}_3)_3$ requires: C, 73.54; H, 5.81; OCH_3 , 30.0.

The non-nitrogenous product is very soluble in chloroform, moderately in acetone and in ether, and sparingly soluble in ethyl alcohol and in ligroin. In each of these solvents the substance shows a blue-violet fluorescence. It is also very slowly soluble in 25% aqueous potash to a yellow-green solution, which becomes pale yellow on dilution. Upon acidification, a distinct turbidity results which/

which can be shaken into ether; the ethereal extract shows a pale blue fluorescence.

With cold concentrated sulphuric acid a deep blue-green colour is produced which gives way to a deep blue on warming; and with cold concentrated nitric acid the substance dissolves to a deep orange-red solution.

The vinyl compound in glacial acetic acid (deep red solution) rapidly absorbed one mole of hydrogen (Adams' catalyst) to give a practically colorless reduction product which turned deep red immediately on contact with air. After filtering off the platinum, the filtrate was diluted with water and extracted with ether; the deep red extracts were dried, evaporated and traces of acetic acid removed in a vacuum desiccator over potash. The remaining small quantity of deep red, viscous residue could not be crystallized, nor could a picrate of it be formed.

Attempts to oxidize the vinyl group to the corresponding carboxylic acid by means of potassium permanganate in rectified acetone were unsuccessful. Despite two experiments, only very small quantities of oily products were obtained which decomposed when an attempt to distil them in a high vacuum was made.

A Hofmann degradation carried out as above with 1.8 gm. O-methyl artabotrine yielded 1.09 gm. (90%) of a clear, syrupy non-nitrogenous product which polymerized over-night to a pale-yellow, horn-like substance. Oxidation of this material with potassium permanganate in rectified acetone yielded a small quantity of crystalline product which has not yet been completely investigated.

13a. Oxidation of artabotrine with potassium permanganate.

Crude artabotrine (2 gm.) was dissolved in very dilute hydrochloric acid and sodium carbonate added until a slight turbidity occurred. A 3% aqueous solution of potassium permanganate was added in volumes of 10 c.c. (= .55 O) at a time. About 14 atoms of oxygen were used up at room temperature; the oxidation was completed on the water bath, requiring 410 c.c. of permanganate or 23 atoms of oxygen in all. The filtered solution was concentrated in vacuo to about 120 c.c., the filtered manganese dioxide added again and dissolved by means of sulphur dioxide. The clear, yellow solution was acidified with concentrated hydrochloric acid and after 43 hours' extraction with ether/

ether in a continuous extractor, the ether was evaporated and the residue dissolved in 100 c.c. boiling water. The hot solution was treated with a solution of calcium acetate until no further precipitation of calcium oxalate occurred. The filtered solution was again acidified with concentrated hydrochloric acid and continuously extracted for 21 hours with ether. Evaporation of the solvent left a crude, crystalline residue weighing 43 mg. which could be crystallized from hot absolute ethyl alcohol in the form of slightly pink, blade-like needles. The colour could not be completely removed by three recrystallizations, m.p. 203-204°.

The substance sublimes without decomposition at 200-225° (15 mm.) in the form of pale-yellow needles.

Found: C, 55.12; H, 3.62; OCH_3 , 24.3; equiv. 239.1.

$\text{C}_9\text{H}_4\text{O}_4(\text{OCH}_3)_2$ requires: C, 55.46; H, 4.20; OCH_3 , 25.2; equiv. 238.0.

After the substance had neutralized one equivalent of alkali in the cold, a known excess of alkali was added, the solution boiled during 10 minutes, cooled and back-titrated. Exactly one more equivalent of alkali was found to have been taken/

taken up; a lactone is therefore indicated.

When one mg. of the substance was heated with an approximately equal amount of resorcinol and one drop of concentrated sulphuric acid in the usual way, and the melt basified with sodium hydroxide, a strong yellow-green fluorescence resulted.

13b. Oxidation of artabotrine with Beckmann's mixture.

Artabotrine (1 gm.) was dissolved in sulphuric acid (4 c.c. of 23%) and during the course of 1 hour at 30-35°, a solution of potassium dichromate (0.32 gm. = 10% excess over 1 oxygen atom) in 2.5 c.c. sulphuric acid (23%) was added. The dark coloured solution was nearly neutralized with sodium carbonate and left in the refrigerator overnight.

The solution was then basified with 2 N. sodium hydroxide and thoroughly extracted with ether, the ethereal extracts being deep red-orange in colour; (sulphur dioxide rapidly discharged this colour). The dried extracts, upon evaporation, yielded a small quantity of ruby-red prisms melting between 172-174°; when mixed with an approximately equal quantity of artabotrine (185-186°) the mixture/

mixture melted at 176-177°. It was subsequently found that the crystals were mainly artabotrine impregnated with the deep colour of the oxidation product. In high vacuum, a colorless sublimate of artabotrine was obtained, whereas the deep colour remained behind largely decomposed.

The original red crystals gave a positive Laubenheimer phenanthraquinone reaction; the final solution, however, must first be basified with sodium hydroxide before being shaken either with ether or chloroform, in order that the basic dyestuff be liberated.

Control experiments carried out in exactly the same way with pukateine and with laurotetanine showed no coloration; the final ethereal extracts were absolutely colorless.

(11) /

(ii) Phenolic fraction (solution A, see page 75).

Carbon dioxide was passed into the alkaline solution containing the phenolic bases until no further precipitation occurred. The voluminous precipitate was dissolved in dilute (2 N.) hydrochloric acid and the solution extracted with ether to remove some coloured matter. The solution was then basified with concentrated ammonia and extracted with ether. After a second passage through acid, the ethereal extracts were dried and concentrated to small volume yielding 3-4 gm. of crystalline material which was identified as artabotrine (mixed melting point).

The green-coloured mother liquor was concentrated to half its volume and after a week deposited a small quantity of another alkaloid in the form of fine, silky needles which were filtered and dried - yield 320 mg. (= 0.0013%). After two recrystallizations from hot methyl alcohol these were obtained as bundles of needles and small prisms melting at 232°.

Found: C, 69.81, 69.85; H, 6.51, 6.55; N, 4.34, 4.27;

OCH_3 , 17.28; $\text{N}(\text{CH}_3)$, 3.90.

$\text{C}_{16}\text{H}_{10}(\text{OH})_2\text{N}(\text{CH}_3)(\text{OCH}_3)_2$ requires: C, 69.73; H, 6.42;

N, 4.28; OCH_3 , 18.95; $\text{N}(\text{CH}_3)$, 4.58.

A Zerewitinoff estimation showed 1.94 equivalents of active hydrogen; two hydroxyl groups require 2.00 equivalents.

The alkaloid is very soluble in chloroform, moderately in methyl alcohol and sparingly soluble in acetone, ether and in benzene. It is also freely soluble in cold 2 N. sodium hydroxide, and with alcoholic ferric chloride a deep purple coloration is produced immediately.

In chloroform, the rotation of the alkaloid was found to be $[\alpha]_D^{15} = +164^\circ$ ($c = 1.22$; $l = 1$ dm).

The alkaloid gives a positive Pellagri test which, according to Gadamer (54) indicates that the para position to the phenolic hydroxyl is unsubstituted.

The alkaloid could be completely methylated as follows: a solution of 30 mg. of the base in 25 c.c. absolute methyl alcohol was treated at 0° with 2 c.c. nitroso N-methyl urethane; 10 drops of methyl alcoholic potash (25%) were added and the solution left in the refrigerator for 18 hours. Another 7 drops of alcoholic potash were then added and after two days in the refrigerator the solution was diluted with slightly acidified water to decompose/

decompose the excess diazomethane and the alcohol removed on the water bath under reduced pressure. The aqueous solution was basified with ammonia and extracted with ether, the extracts in turn being washed with small portions of 1 N. sodium hydroxide to remove unmethylated phenolic alkaloid. After being washed with water, the ethereal solution was dried and evaporated, yielding 27 mg. of a viscous oil which was difficult to crystallize.

The product was taken up in 2-3 c.c. of methyl alcohol, treated with a few drops of methyl iodide and left overnight. The methiodide was precipitated by the addition of dry ether, and after recrystallization from methyl alcohol clusters of needles were obtained which melted at 245-246° with decomposition.

Found: OCH_3 , 25.70.

$\text{C}_{18}\text{H}_{18}\text{N}(\text{OCH}_3)_4\text{I}$ requires OCH_3 , 24.95.

A mixture of this substance with an approximately equal amount of the methiodide of O-methyl artabotriline (253°) melted at 248-249°; thus it is probable that the two substances are identical.

(iii) /

(iii) Non-phenolic alkaloid from precipitate A
(see page 74).

Upon close examination the crystalline precipitate A proved to be the hydrochloride of a new, non-phenolic alkaloid. The free base, liberated from the hydrochloride, is a yellow, varnish-like substance which could not be crystallized. The hydrochloride, on the other hand, is readily purified by carefully adding dilute hydrochloric acid to its solution in water. The salt, because of its sparing solubility, soon separates out in very fine, colourless needles. After two such recrystallizations, the hydrochloride was washed with small amounts of ethyl alcohol and ether and dried, m.p. $273-274^{\circ}$ with decomposition.

Found: C, 65.17, 65.14; H, 5.40, 5.50; N, 4.36; Cl, 10.69; OCH_3 , 9.60.

$\text{C}_{16}\text{H}_{11}(\text{NH})(\text{OCH}_3)(\text{CH}_2\text{O}_2).\text{HCl}$ requires: C, 65.16; H, 5.43; N, 4.22; Cl, 10.71; OCH_3 , 9.35.

No N-methyl group could be detected by the method of Herzig-Meyer; the alkaloid, however, readily forms a nitroso derivative, and the Gaebel test for the presence of a methylene dioxy group proved/

proved to be positive.

A Zerewitinoff estimation on the salt showed 1.98 reactive hydrogen atoms; one imino group and the salt-hydrogen together require 2.00 active hydrogens.

The solubility of the hydrochloride was found to be approximately 0.5 gm. in 100 c.c. warm water (50-60°).

The free base in chloroform shows a rotation of $[\alpha]_D^{17} = -18.9^\circ$ ($c = 2.69$; $l = 1$ dm), whereas that of the hydrochloride in absolute ethyl alcohol was found to be $[\alpha]_D^{18} = -41.8^\circ \pm 4.18^\circ$ ($c = 0.24$; $l = 1$ dm).

Nitroso-derivative of free base (artabotrinine).

By adding a cold, saturated, aqueous solution of sodium nitrite to one of the base in dilute acetic acid, the nitroso-derivative soon separated out as a buff-coloured crystalline precipitate. After two recrystallizations from ethyl alcohol, clusters of small, tan, hexagonal plates of m.p. 203-204° were obtained.

Found: N, 8.23.

$C_{18}H_{16}O_4N_2$ requires: N, 8.62.

Methylation/

Methylation of artabotrinine to N-methyl artabotrinine (55, 56).

The free base (0.6 gm. = 1 mol) was refluxed at 100° for two hours with 1.2 mols (0.48 gm.) of formic acid (25% solution) and 2.4 mols (0.38 gm.) of formaldehyde (40% solution) in 10 c.c. of water. The cooled solution was basified with concentrated ammonia and extracted with ether which, after being dried and evaporated, left behind a semi-crystalline residue consisting of tertiary and unmethylated secondary base. To separate the two, the residue was dissolved in freshly distilled acetic anhydride (4.5 c.c.) and left at room temperature over-night. Water (30 c.c.) and concentrated hydrochloric acid (3 c.c.) were added and after complete decomposition of the acetic anhydride, the solution was repeatedly extracted with ether. The ethereal solution was washed several times with dilute (1 N.) sodium hydroxide, dried and evaporated, yielding about 30 mg. of an oily N-acetyl compound which was not inclined to crystallize.

The solution after the removal of N-acetyl artabotrinine was basified with sodium hydroxide and extracted with ether. After being dried and evaporated this left 0.276 gm. of a crystalline substance/

substance which after two recrystallizations from diluted alcohol was obtained as colourless, blade-shaped needles melting at 132-133°.

Found: C, 74.09; H, 6.01; N, 4.71.

$C_{19}H_{19}O_3N$ requires: C, 73.78; H, 6.18; N, 4.53.

N-methyl artabotrinine is isomeric with O-methyl pukateine (m.p. 137°); however, a mixture of approximately equal quantities of the two substances melted at 98-99°.

In absolute alcohol, N-methyl artabotrinine shows a rotation of $[\alpha]_D^{16} = -53.8^\circ$ (c = 0.424; l = 1 dm).

N-methyl artabotrinine forms a methiodide with methyl iodide in methyl alcohol which, after two recrystallizations from methyl alcohol-ether was obtained as small, silky needles of m.p. 223-224°.

Hydrolysis/

Hydrolysis of the methylene dioxy group. (58)

Artabotrinine hydrochloride (0.5 gm.), phloroglucinol (0.7 gm.) and 11 c.c. of 40% sulphuric acid were heated to gentle boiling for 5 minutes and the reaction mixture afterwards heated in a boiling water bath for 16 hours. Approximately 2 volumes of boiling water were added, the bulky precipitate of deep red phloroglucinol condensation products filtered off and the precipitate washed several times with small portions of boiling water, the washings being added to the main filtrate. The orange-coloured aqueous solution was extracted continuously for 16 hours with ether; it was then basified with concentrated ammonia and rapidly extracted with ether. The ethereal solution was dried over sodium sulphate and concentrated to small volume; the last traces of solvent were removed over paraffin wax in a desiccator from which the air had been displaced by carbon dioxide. The pale tan-coloured dihydroxy compound was amorphous and weighed ca. 50 mg. It gave only a slight precipitate with Mayer's reagent; alcoholic ferric chloride produced a deep blue-green coloration, and Fehling's solution was readily/

readily reduced by it.

The dihydroxy compound reacts violently with nascent diazomethane in absolute methyl alcohol at 0°. Half of the material was lost when one methylation experiment got out of hand and blew up with a loud detonation.

The methylation product was worked up in the usual way, and the ethereal solution washed with dilute sodium hydroxide to remove unmethylated base. The ethereal solution was dried and evaporated, leaving behind an oily non-phenolic base which could not be crystallized, and various attempts to prepare salts of it were likewise unsuccessful. In acid solution, the substance gave a heavy precipitate with Mayer's reagent. Because of the small quantity of material at hand, purification was not possible.

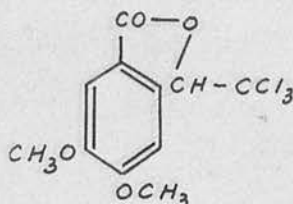
Found: OCH_3 , 22.45.

$\text{C}_{18}\text{H}_{12}\text{N}(\text{OCH}_3)_3$ requires: OCH_3 , 29.90.

Synthetic Experiments.

a. Synthesis of 3-4 dimethoxy trichloromethyl phthalide.(59)

3-4-Dimethoxy benzoic acid
(60) (16.6 gm.), chloral (41.8
gm.) and concentrated sulphuric
acid (45 c.c.) were shaken



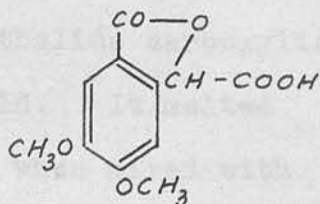
together for three days, and the
reaction mixture poured over ice, when an almost
colourless sludge separated out. After filtration
the precipitate was washed with a little water and
then taken up in an excess of 2 N. sodium carbonate.
This treatment caused the solution of practically
the whole of the precipitate, the small quantity
of insoluble material being shaken with ether. The
ethereal solution was washed several times with 2 N.
sodium hydroxide to decompose a small amount of
chloral; it was then washed with water and dried,
yielding ca. 0.9 gm. of colourless crystals melting
at 144° (146° in the literature).

From the combined alkaline solutions, 10 gm.
of 3-4 dimethoxy benzoic acid were recovered.

Hydrolysis/

Hydrolysis of the trichloro compound to 3-4 dimethoxy phthalide carboxylic acid. (59)

By digesting 0.2 gm. of the above product with 40 c.c. of 20% sodium hydroxide at 100° for two and a half hours, the substance gradually went into solution. The cooled solution was extracted with ether to remove a small quantity of undissolved material, then strongly acidified with concentrated hydrochloric acid and repeatedly extracted with ether. After being dried and evaporated the ethereal solution left behind 70 mg. of the desired acid in the form of stout prisms melting at 206-207° with decomposition (recorded m.p. 207° and 211° depending upon rate of heating).



When this acid was mixed with an approximately equal quantity of the natural acid (m.p. 203-204°) obtained from the oxidation of artabotrine with potassium permanganate, the mixture melted at 175°. The acids are therefore not identical.

The synthetic acid did not give a positive fluorescein reaction when treated with resorcinol and sulphuric acid in the usual way.

b. By condensing 2-4 dimethoxy benzoic acid (61) with chloral and sulphuric acid as outlined above, followed by the hydrolysis of the resulting product, the corresponding 2-4 dimethoxy phthalide carboxylic acid was obtained in very poor yield. It melted at 149-150° with decomposition and when mixed with an approximately equal quantity of the natural acid (203-204°) the mixture melted at 137°, thus showing that the acids are not identical.

The synthetic acid did not give a positive fluorescein reaction in the usual way.

c. Condensation of 2-5 dimethoxy benzoic acid (62) with chloral and sulphuric acid, and subsequent hydrolysis of the product as outlined above, afforded the corresponding 2-5 dimethoxy phthalide carboxylic acid, also in poor yield. It melted at 186-187° with decomposition and when mixed with an approximately equal quantity of the natural acid (203-204°) the mixture melted at 167-168°; the two substances are therefore not identical.

This synthetic acid also failed to respond to the fluorescein test.

Because/

Because of the extremely poor yields in which they were obtained, synthetic acids (b) and (c) are being prepared in greater quantity in order that their constitutions may be proved.

Ultra-violet Absorption Spectra.

The technique employed in this work was similar to that of Girardet (52), the spectrographic measurements were made with a Bellingham and Stanley spectrograph giving a spectrum of 120 mm. from $\lambda 4240$ to $\lambda 2340$. The substances examined were purified by several recrystallizations and dissolved in purified 98% ethyl alcohol. Solutions of M/10,000 were placed in a quartz Baly tube and exposed to an iron arc for a period of 60 seconds, the thickness of the solution varying from 5 to 60 mm. Ilford, thin-film half-tone plates were used.

The Crystallography of Artabotrine.

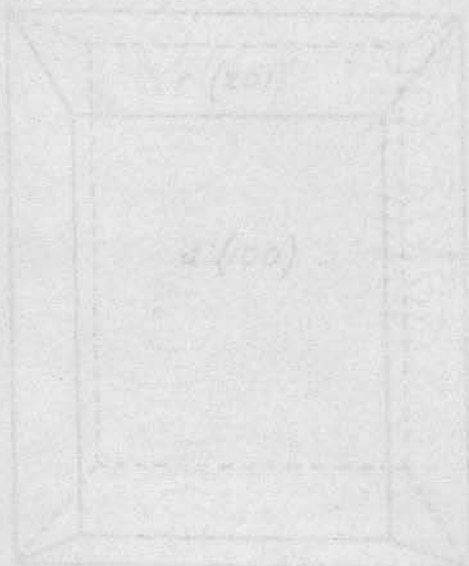
C.J. Brown and E.G.Cox.

Artabotrine crystallises in large tabular orthorhombic crystals (see figure on page 111), exhibiting the forms $\underline{a}\{100\}$, $\underline{m}\{210\}$ and $\underline{r}\{201\}$. ($\underline{m} : \underline{a} = 49^{\circ}46'$, $\underline{r} : \underline{a} = 56^{\circ}27'$). Measurements of X-ray rotation photographs gave for the cell dimensions $[\underline{a}] = 23.13$, $[\underline{b}] = 9.89$ and $[\underline{c}] = 7.51$ A. and the density was found by flotation to be 1.29 g./c.c. so that there are four molecules of $\text{C}_{20}\text{H}_{23}\text{O}_4\text{N}$ in the unit cell. (M.W. by X-rays, 332; calc. 341). From the analysis of a series of X-ray oscillation photographs the space-group was found to be $P2_12_12_1$ so that the possibility of the cell containing two molecules of $\text{C}_{40}\text{H}_{46}\text{O}_8\text{N}_2$ is excluded.

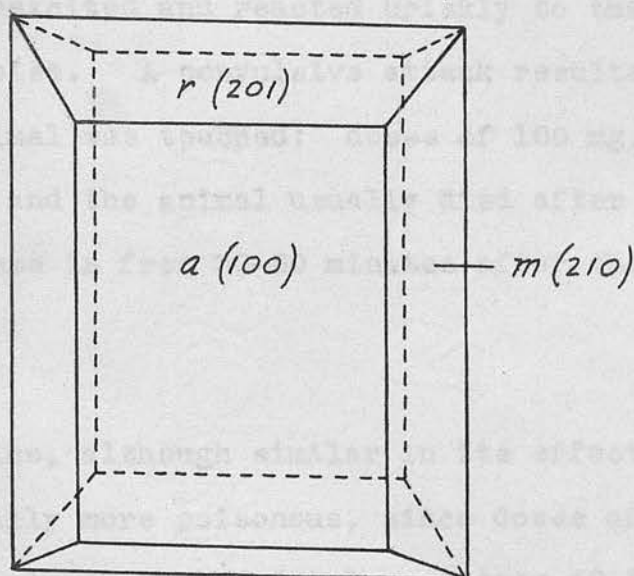
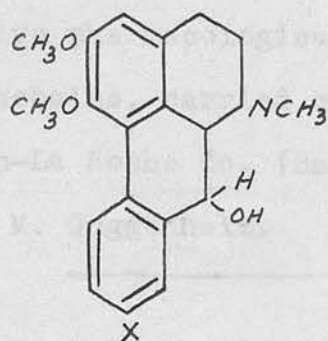
The refractive indices (for sodium light) are $\alpha = 1.50$ (approx.), $\beta = 1.67$ and $\gamma = 1.75$. $[\underline{a}]$ is the acute bisectrix and $\underline{c}(001)$ the plane of the optic axes. The optic axial angle $2V$ is 70° approx.

From the above results it appears probable that the longest direction of the molecules is approximately parallel to $[\underline{b}]$ and that the planes of the molecules/

molecules are more nearly parallel to $a(100)$ than to $c(001)$. On account of the relatively short length of the $[b]$ axis it seems unlikely that the third methoxyl group is in the position X (formula, page 111).



ARTABOTRINE



ARTABOTRINE

Comparative pharmacological tests of artabotrine with thebaine, carried out in the laboratories of the Hoffmann-La Roche Co. (Basel), through the courtesy of Dr M. Guggenheim.

(i) Artabotrine was intravenously injected in a mouse in a dose of 50 mg./kg., and the following symptoms were observed: after first being accelerated, respiration became spasmodic and 30 minutes after the injection the animal was distinctly overexcited and reacted briskly to the slightest noise. A convulsive attack resulted when the animal was touched; doses of 100 mg./kg. were fatal, and the animal usually died after convulsive spasms in from 30-60 minutes after the injection.

(ii) Thebaine, although similar in its effects, was essentially more poisonous, since doses of 7.5 mg./kg. always proved fatal. A dose of 5 mg./kg. was fatal to one out of four animals. Toxic symptoms, in the form of accelerated respiration, shivering and strong excitability, could be observed with doses of 1 mg./kg. Higher doses produced convulsive stretching of the limbs and death usually/

usually occurred during such convulsive attacks.

The pharmacologist's conclusions were as follows: Artabotrine possesses toxic properties which are distinctly similar to those of thebaine.

and has been shown to be, in addition to the principal alkaloid "artabotrine", two other alkaloids, viz., "thebaine" and "artabotrinine".

From a detailed study of artabotrine, the probable structural formula has been determined and much data has been accumulated regarding the structures of the other two alkaloids are also included.

Artabotrine

1. From analytical data obtained with the purified base as well as with various derivatives, the empirical formula $C_{21}H_{23}O_2N$ is preferred to that of $C_{21}H_{25}O_2N$.

2. The previously unknown function of the fourth oxygen atom in artabotrine has been determined and shown to be due to an alcoholic group possessing slightly acidic properties. The hydroxyl group

(f) Summary.

The bark of the Philippine species of Artabotrys suaveolens, Blume, has been re-examined and has been shown to yield, in addition to the principal alkaloid "Artabotrine", two new alkaloids, viz., "Suaveoline" and "Artabotrinine".

From a detailed study of artabotrine, the probable structural formula has been determined; and such facts as have been ascertained regarding the structures of the other two alkaloids are also included.

a. Artabotrine.

1. From analytical data obtained with the purified base as well as with various derivatives, the empirical formula $C_{20}H_{23}O_4N$ is preferred to that of $C_{21}H_{25}O_4N$.

2. The previously unknown function of the fourth oxygen atom in artabotrine has been determined and shown to be due to an alcoholic group possessing slightly acidic properties. The hydroxyl group can/

can be methylated either with nascent diazomethane or by means of dimethyl sulphate; in the latter case the nitrogen atom is also methylated.

3. The product obtained from the action of chlor-ethyl carbonate on artabotrine was neutral and optically inactive; a tetrahydro-isoquinoline ring system is therefore indicated.

4. The first stage of the Hofmann degradation yielded a crystalline, optically active, unsaturated methine-base. Further methylation of the methine base and treatment with alkali resulted in the elimination of trimethylamine, and in the formation of a non-nitrogenous vinyl compound which exhibited properties similar to those of 10-phenanthrol.

The elimination of trimethylamine after the second methylation indicates that the nitrogen belongs to one ring.

That a vinyl group was present was shown by the rapid absorption of one mol of hydrogen, but owing to the instability of ~~of~~ the resulting compound, it could not be isolated.

Attempts to oxidize the non-nitrogenous vinyl compound to the corresponding carboxylic acid were unsuccessful.

5. Oxidation of artabotrine with chromic acid yielded a small quantity of deep coloured substance with properties resembling those of an ortho-quinone.

6. Oxidation of artabotrine with aqueous potassium permanganate yielded a small amount of a lactone acid which possesses two methoxyl groups. In an attempt to synthesize the substance, three isomeric lactone acids were prepared but found not to be identical with the natural acid.

7. The ultra-violet absorption spectra of artabotrine has been found to be similar to that of pukateine.

8. The crystallographic analysis of artabotrine has contra-indicated a double-molecule structure for the base.

9. The pharmacological activity of artabotrine has been re-examined and compared with that of thebaine.

b. /

b. Suaveoline.

1. The empirical formula of this new phenolic alkaloid has been shown to be $C_{18}H_{21}O_4N$.
2. The alkaloid is optically active and possesses two methoxyl groups, one N-methyl group, and a Zerewitinoff estimation showed two active hydrogen atoms.
3. The alkaloid gives a positive Pellagri reaction, indicating that the para position to the phenolic hydroxyl group is probably free.
4. The alkaloid can be completely methylated with nascent diazomethane to an oily base which readily forms a crystalline methiodide. A mixed melting point has indicated that this methiodide is probably identical with that obtained from fully methylated artabotrine.

c. /

c. Artabotrinine.

1. This new, non-phenolic alkaloid possesses the empirical formula $C_{18}H_{17}O_3N$, and is amorphous.
2. The alkaloid is an optically active secondary base, and possesses one methoxyl as well as a methylene dioxy group; the latter two groups therefore account for all three oxygen atoms. A Zerewitinoff estimation showed one active hydrogen.
3. The alkaloid can be methylated to the corresponding crystalline N-methyl derivative by means of formaldehyde and formic acid.
4. N-methyl artabotrinine is isomeric, but not identical, with O-methyl pukateine.
5. The methylene-dioxy group was hydrolysed by means of phloroglucinol and sulphuric acid and the resulting dihydroxy compound methylated with nascent diazomethane.
6. The ultra-violet absorption spectra of artabotrinine hydrochloride has been examined and compared with that of dicentrine.

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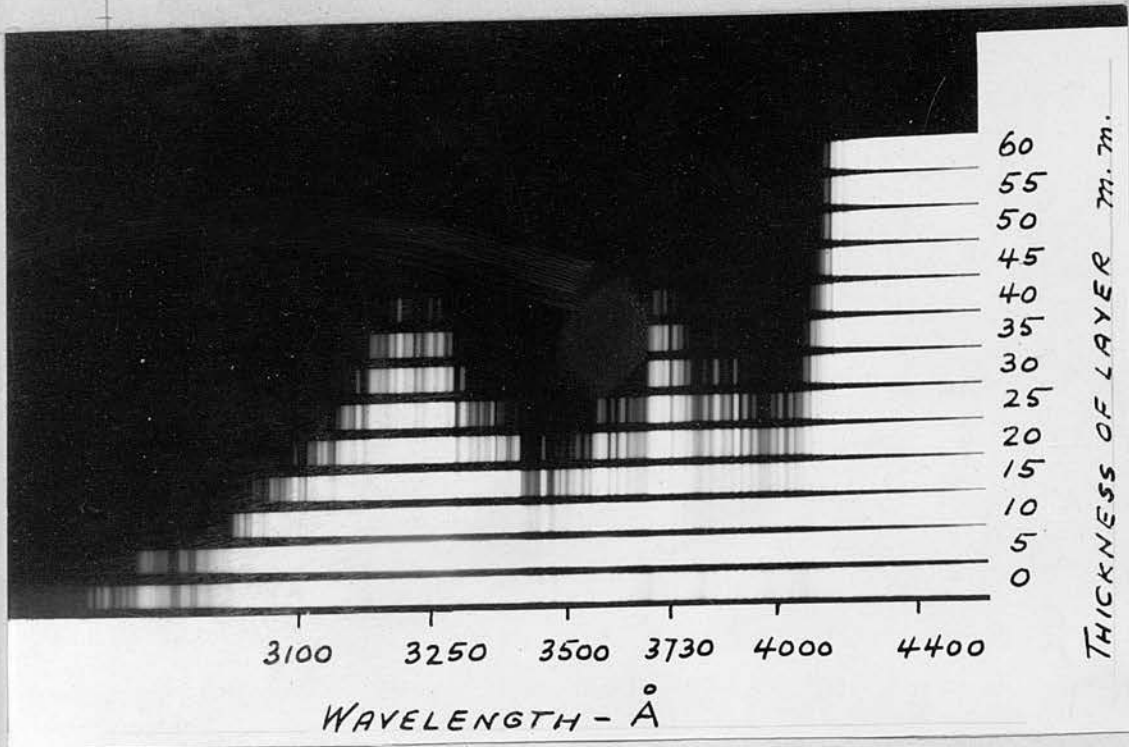
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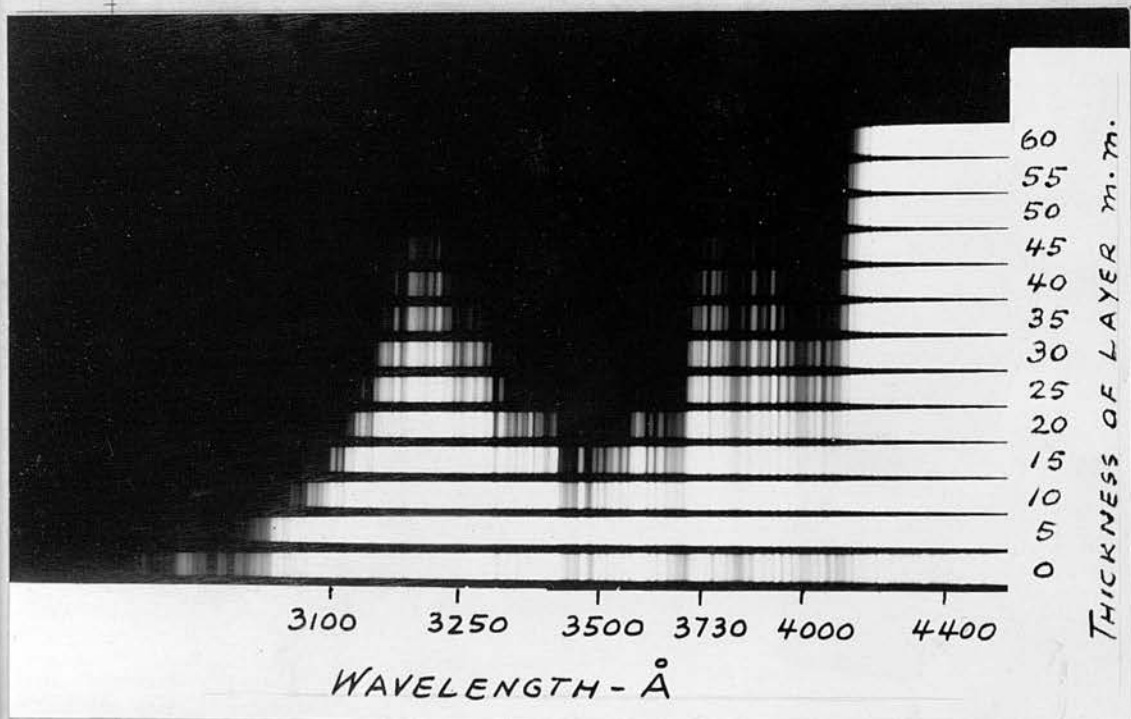
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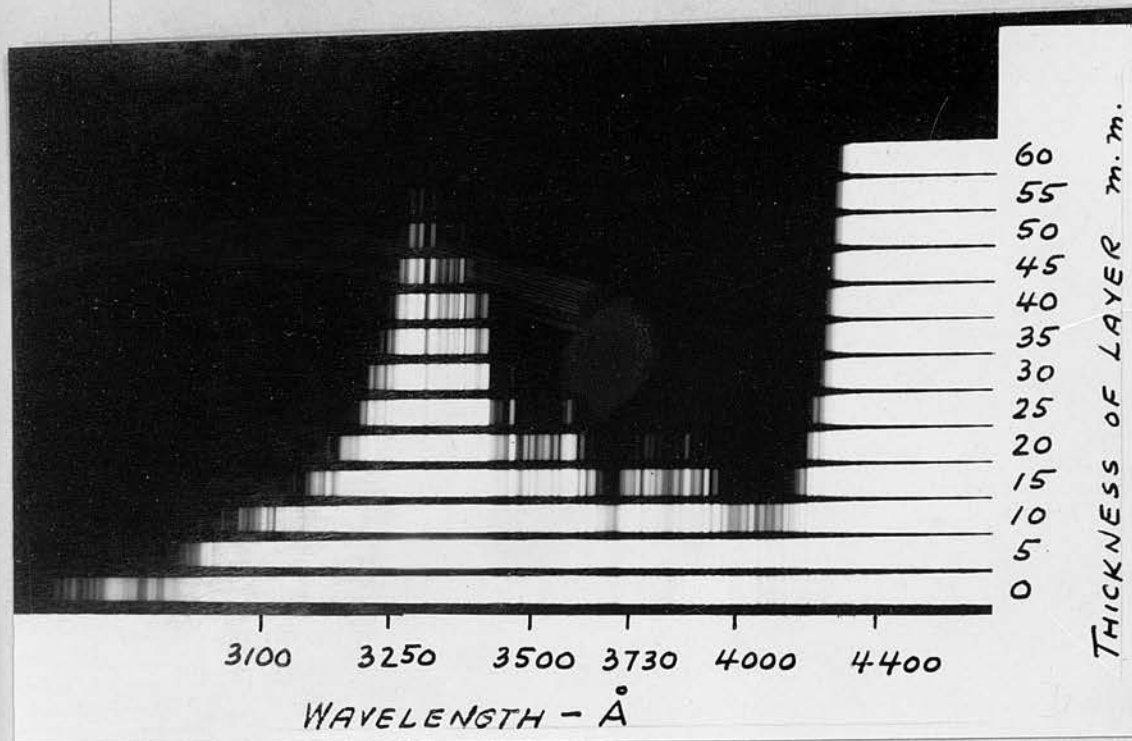
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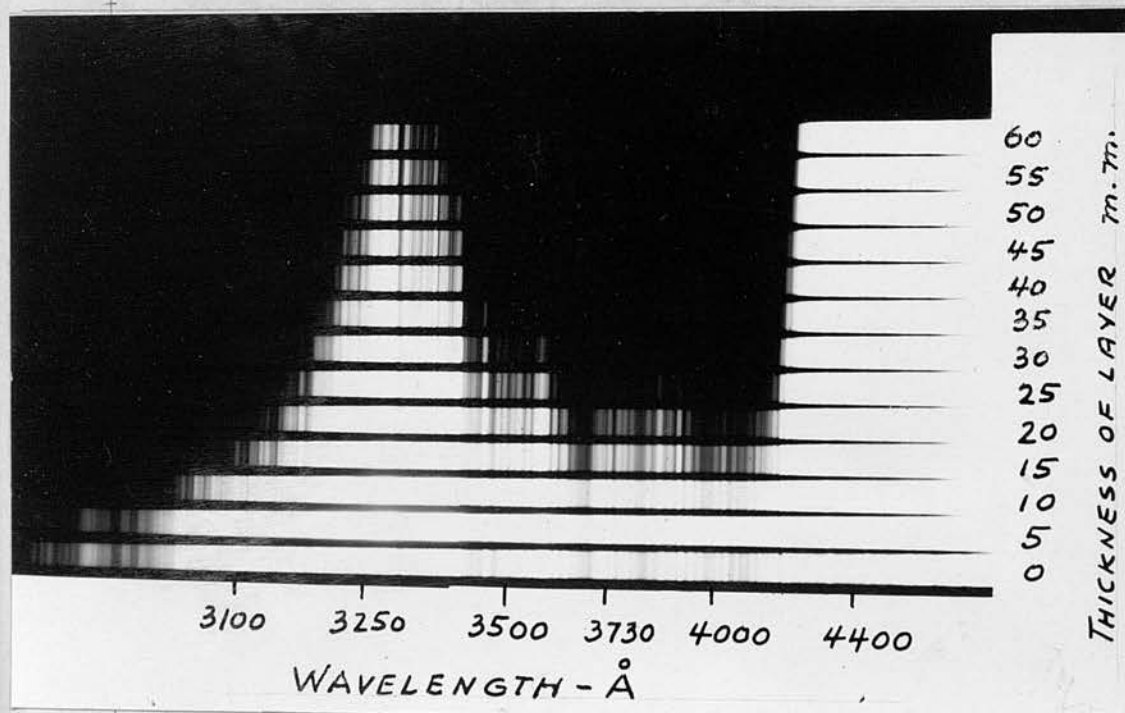
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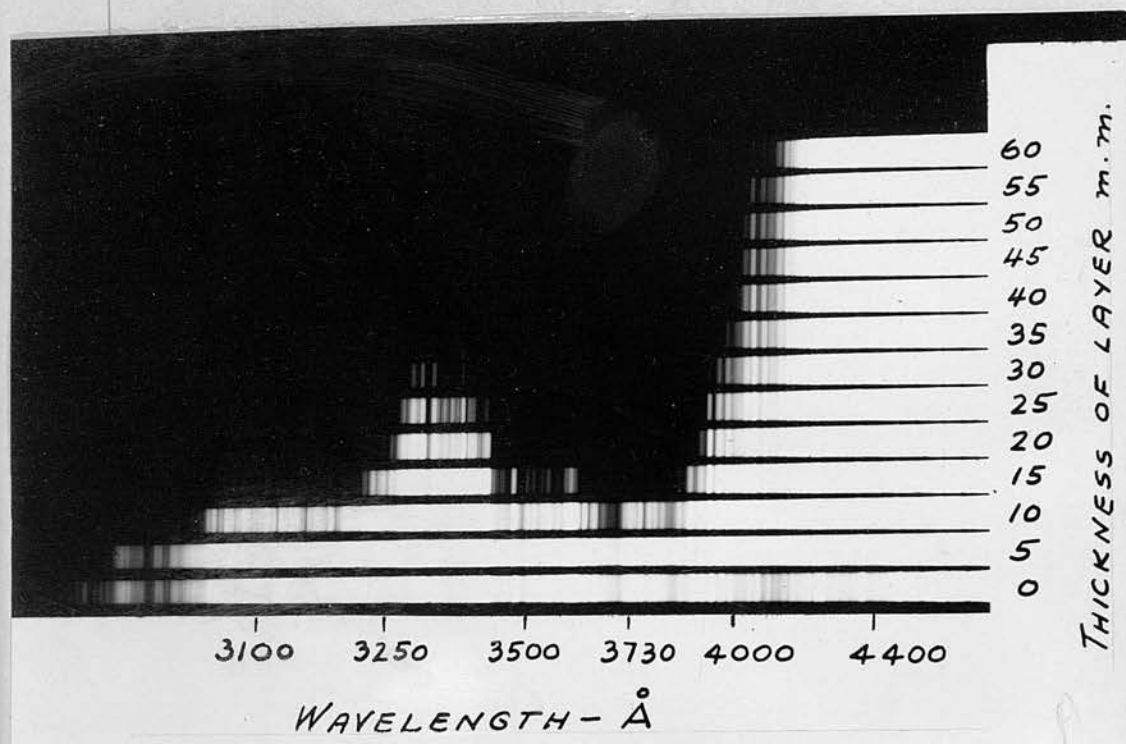
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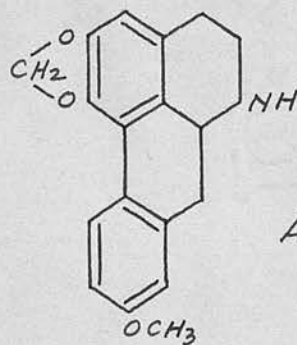
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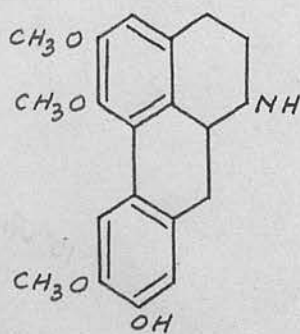
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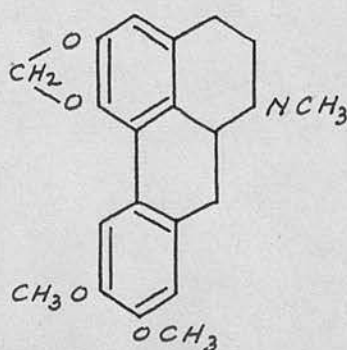
ARTABOTRINE HYDROCHLORIDE



ARTABOTRINE ?



LAUROTETANINE



DICENTRINE